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John Van horne Constable

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**Photosynthetic response to elevated CO₂ concentrations in the
aerenchyma of *Typha latifolia* L. leaves**

Constable, John Van Horne, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1993

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PHOTOSYNTHETIC RESPONSE TO ELEVATED CO₂ CONCENTRATIONS
IN THE AERENCHYMA OF TYPHA LATIFOLIA L. LEAVES

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

The Department of Botany

by

John V.H. Constable
B.S., Syracuse University, 1984
May 1993

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ABSTRACT

This study examined the importance of the leaf aerenchyma gas space as a CO₂ source for photosynthesis in Typha latifolia L. (broadleaf cattail). In the field there was a distinct diurnal pattern of CO₂ concentration ([CO₂]) in the aerenchyma gas space. At dawn the aerenchyma [CO₂] was 4 to 18 times above atmospheric levels. By midday the aerenchyma [CO₂] declined to near atmospheric levels and increased again in the late afternoon. It is hypothesized that this diurnal pattern may be controlled by photosynthetic demand for CO₂. Aerenchyma gas space was estimated as >50% of leaf volume, and the continuity of the aerenchyma gas space through the rhizome-shoot transition was confirmed using tracer dyes. Anatomical examination revealed that the aerenchyma gas space separates the anatomically similar adaxial and abaxial palisades. Each palisade was exposed to two CO₂ sources: (1) atmospheric CO₂ diffusing through the epidermal stomata along a gaseous pathway; and (2) aerenchyma gas space CO₂ diffusing through the cells of the internal surface along an aqueous pathway. Using gas exchange measurements, net photosynthetic CO₂ uptake rate (P_N) of isolated adaxial and abaxial palisades of intact leaves was 6.0 and 4.0 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, and saturated at a photosynthetic photon flux density of 900 and 700 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, respectively. P_N response to [CO₂] was similar for intact leaves and dissected leaves when [CO₂]

in aerenchyma gas space was held constant. At a constant epidermal $[CO_2]$ of about $350 \mu L \cdot L^{-1}$, internal P_N from the aerenchyma gas space increased linearly with $[CO_2]$ to $1.92 \mu mol \cdot m^{-2} \cdot s^{-1}$ at about $900 \mu L \cdot L^{-1}$, the highest $[CO_2]$ used. Over the same range of aerenchyma gas space $[CO_2]$ epidermal P_N declined 69%. These results indicate that CO_2 can be assimilated from both the atmospheric and aerenchyma gas space CO_2 sources and that these sources of CO_2 could be "competitive." Although internal P_N measured in the laboratory is low, at aerenchyma gas space $[CO_2]$ s found in the field, internal P_N could represent a significant carbon source for cattail.

CHAPTER 1

Literature Review

INTRODUCTION

This dissertation is divided into five chapters that include the introduction, a characterization of cattail internal leaf CO_2 concentrations ($[\text{CO}_2]$) in the field, laboratory measurements of the response of leaf P_N (net rate of photosynthetic CO_2 uptake) to photosynthetic photon flux density (PPFD), laboratory measurements of leaf P_N response to atmospheric and aerenchyma gas space $[\text{CO}_2]$, and a final chapter presenting conclusions of the study.

The introduction will review the effect of $[\text{CO}_2]$, both atmospheric and internal, on plant P_N . A brief review of the biochemistry of carbon fixation will be presented first. Second, experimental studies on the effects of increased atmospheric $[\text{CO}_2]$ on plant P_N and growth are surveyed. Third, the potential importance of an elevated internal $[\text{CO}_2]$ in plant tissues is discussed. Gas transport in the aerenchyma tissue of aquatic plants is reviewed next. The final section introduces Typha latifolia L. (cattail) as a model system for estimating the significance of an internal CO_2 source for plant growth and outlines the dissertation research objectives.

A BRIEF OVERVIEW OF PHOTOSYNTHETIC BIOCHEMISTRY

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; E.C. 4.1.1.39) catalyzes the primary photosynthetic carboxylation reaction in plants. The first stable products of C_3 photosynthesis are two molecules of 3-phosphoglyceric acid (3-PGA) resulting from the addition of CO_2 to ribulose-1,5-bisphosphate (RuBP). Rubisco evolved at a time in the earth's history when the atmosphere had high CO_2 and low O_2 concentrations (Böger 1980) and perhaps as a result Rubisco has a relatively low affinity for CO_2 ($K_m(CO_2) = 8 - 25 \mu M$; Bowes 1991). This $K_m(CO_2)$ may explain Rubisco's abundance in leaves where it accounts for up to 50% of the total soluble protein (Bowes 1991). Using ATP and NADH produced by PPFD absorption, 3-PGA is reduced to triose phosphate which can regenerate RuBP, produce starch or be exported from the chloroplast. In competition with the carboxylase reaction is an oxygenase reaction with O_2 having a much lower affinity relative to CO_2 ($K_m(O_2) = 350 - 650 \mu M$; Bowes 1991). Oxygenation of RuBP produces phosphoglycolate, the starting point for photorespiration, or the C_2 cycle (Husic et al. 1987). The C_2 cycle requires energy and releases previously assimilated CO_2 , thus representing an energy and carbon loss for the plant. Because of this competition with O_2 , net carbon gain is reduced between 15% and 50% (Ogren 1984; Gerbaud and André 1987; Husic et al. 1987;

Sharkey 1988). While the physiological function of the C_2 cycle is debated, it is generally considered a detrimental process from the view point of carbon acquisition. The rate at which carbon is fixed by Rubisco is a function of the K_m and V_{max} of the competing carboxylase and oxygenase reactions, and the ratio of the CO_2 to O_2 concentrations (Ogren 1984).

The present CO_2/O_2 ratio in the earth's atmosphere is approximately 0.0017. Conditions that increase the CO_2/O_2 ratio favor carboxylase activity relative to oxygenase activity. In some species, the evolution of structural and biochemical characteristics has produced an increase in the CO_2/O_2 ratio at the site of Rubisco activity. The two best studied systems are C_4 photosynthesis and the inorganic carbon concentrating mechanism found in certain algae. C_4 plants have two photosynthetic carboxylation reactions. The first reaction involves the carboxylation of phosphoenolpyruvate (PEP) to produce organic acid, and the second reaction results in carboxylation of RuBP by Rubisco forming 3-PGA. In mesophyll cells of C_4 plants, HCO_3^- is formed from dissolved atmospheric CO_2 and is used to carboxylate PEP by PEP carboxylase and form oxaloacetic acid (OAA). OAA is quickly converted to an organic acid, either aspartate or malate, depending on the particular species (Ogren 1984). These organic acids are transported to the photosynthetic bundle sheath cells and are

decarboxylated, releasing CO_2 . The release of CO_2 within the bundle sheath cells concentrates CO_2 around Rubisco. This CO_2 concentrating mechanism is thought to produce $[\text{CO}_2]$ s nearing 1% in the bundle sheath cells (Bowes 1991), producing a CO_2/O_2 ratio of approximately 0.0476, which favors RuBP carboxylation and minimizes RuBP oxygenation.

The second mechanism of concentrating CO_2 is the dissolved inorganic carbon ($\text{CO}_2 + \text{HCO}_3^- + \text{CO}_3^{2-}$; DIC) concentrating mechanism (Moroney and Tolbert 1985). This mechanism is found in cyanobacteria and certain green microalgae (Badger 1987) which appear to possess the same C_3 photosynthetic pathway as higher plants. The concentrating mechanism is environmentally regulated based on the availability of external DIC. At high external DIC these organisms have a relatively low CO_2 affinity and significant photorespiration as do typical C_3 plants. In contrast, the $K_m(\text{CO}_2)$ declines to 0.4 - 3 μM at low external $[\text{CO}_2]$ (Moroney and Tolbert 1985). During adaptation to low DIC conditions, transcription of carbonic anhydrase and additional proteins is induced (Manuel and Moroney 1988). While the exact mechanism of regulation is unclear, the inter-conversion of CO_2 and HCO_3^- by an extracellular (Kimpel et al. 1983) and/or intracellular carbonic anhydrase is essential (Moroney et al. 1987; Husic et al. 1988). This biochemical adaptation can increase the $[\text{CO}_2]$ in the chloroplast 50 to 1000-fold (Badger 1987)

relative to the external environment, producing CO_2/O_2 ratios between 0.0833 and 1.6667, which are favorable to RuBP carboxylation.

The ability of some species to concentrate CO_2 and reduce photorespiration by Rubisco leads to increased carbon gain and growth. In contrast many C_3 species, including T. latifolia, are dependent on the ambient CO_2/O_2 ratio and experience significant carbon loss due to the C_2 cycle.

THE EFFECT OF ELEVATED $[\text{CO}_2]$ ON PHOTOSYNTHESIS

In most plants Rubisco functions at ambient atmospheric concentrations of oxygen and carbon dioxide. Increasing the CO_2/O_2 ratio by experimentally increasing the $[\text{CO}_2]$ to a constant and uniform level has produced a broad range of responses in field and laboratory experiments (see reviews by Bowes 1991; and Stitt 1991). Many of these studies were designed to evaluate the potential effects of the rising atmospheric $[\text{CO}_2]$ which is expected to double in the next century.

Response to elevated atmospheric $[\text{CO}_2]$ depends on whether plants are C_3 , C_4 or CAM. Cure and Acock (1986) reviewed the effects of elevated $[\text{CO}_2]$ on crop species with both C_3 and C_4 photosynthetic pathways and concluded that productivity of C_3 crops will increase up to 30% if the atmospheric $[\text{CO}_2]$ doubles. In C_4 species the response to

elevated atmospheric $[CO_2]$ will be less than C_3 species because they effectively concentrate CO_2 around Rubisco. However, C_4 photosynthetic rate may increase somewhat due to fixation of CO_2 that diffuses directly to the bundle sheath chloroplasts, bypassing the C_4 cycle (Edwards and Black 1971; Ray and Black 1979). C_3 and C_4 species have similar dark respiration rates (Byrd et al. 1992), therefore, the reduced dark respiration rate of C_3 species at elevated atmospheric $[CO_2]$ may also apply to C_4 species (Amthor et al. 1992). It is postulated that productivity of CAM species will increase 1% for each $10 \mu L \cdot L^{-1}$ rise in ambient $[CO_2]$ (Nobel and Hartsock 1986). Biomass of all plants, regardless of photosynthetic pathway, should increase as atmospheric $[CO_2]$ increases, but C_3 species should respond more significantly than C_4 or CAM species (Potvin and Strain 1985; Smith et al. 1987).

While photosynthetic biochemistry exerts a controlling force on the response to elevated $[CO_2]$, the final response can be modified by many factors including light environment, nutritional status and the plant developmental stage. Sionit et al. (1982) concluded that elevation of both $[CO_2]$ and light intensity increased dry matter production to a greater extent than either factor alone. The increase was evident at all stages of growth, but was most dramatic during early growth because of the high photosynthetic rates of young leaves. Nitrogen deficiency

can minimize plant response to elevated $[\text{CO}_2]$ (Larigauderie et al. 1988).

Although most evidence suggests a rise in plant productivity with an increase in atmospheric $[\text{CO}_2]$, several experiments have shown counter results. DeLucia et al. (1985) found elevated $[\text{CO}_2]$ caused only a transient increase in carbon gain as photosynthetic rate per unit area declined due to non-stomatal factors, either feedback inhibition and/or chloroplast structural damage by starch accumulation. Similar results were obtained at twice atmospheric $[\text{CO}_2]$ for Eichhornia crassipes (Mart.) Solms, a non-rooted aquatic species. After four weeks of elevated $[\text{CO}_2]$ plants had half the photosynthetic rate of ambient $[\text{CO}_2]$ plants (Spencer and Bowes 1986). These findings imply that after a short-term rise in carbon gain the photosynthetic apparatus may become damaged and have a rate of net carbon gain below that of the control plants. A decline in photosynthetic rate per unit area could be offset by a decrease in root/shoot ratio as leaf area increases (Sionit et al. 1982; Cure and Acock 1986; Spencer and Bowes 1986). Therefore, control plants and plants acclimated to elevated $[\text{CO}_2]$ may have similar rates of actual carbon gain.

The elevation of $[\text{CO}_2]$ influences a range of metabolic processes other than P_n , and alters anatomy and morphology. Dark respiration rates in herbaceous annuals and perennials

can decrease approximately 15% at elevated $[\text{CO}_2]$ (Bunce 1990; Bunce and Caulfield 1991). Dark respiration was reduced 35 - 55% at $700 \mu\text{L}\cdot\text{L}^{-1}$ in woody perennials with the greatest effects occurring early in the season when leaf tissue was the youngest (Bunce 1992). Elevation of atmospheric $[\text{CO}_2]$ can also alter partitioning of carbon resources between metabolic processes (see review by Allen 1990) or allocation of carbon to processes such as seed production (Havelka et al. 1984). Common morphological responses to elevated $[\text{CO}_2]$ include increases in stem length and branching, and increases in leaf area, leaf thickness, leaf area duration and root-shoot ratio (Sionit et al. 1981a; Larigauderie et al. 1988). Plants grown at elevated $[\text{CO}_2]$ also can have a greater water-use efficiency and drought resistance than plants grown at ambient $[\text{CO}_2]$ (Sionit et al. 1981b; Morison and Gifford 1984). These changes demonstrated by laboratory experiments could affect the ecological relationships of these plants in the field.

Results of laboratory experiments can be difficult to extrapolate to the field where the response to elevated $[\text{CO}_2]$ is integrated with responses to other environmental factors. In big bluestem, a C_4 species, Kirkham et al. (1991) found that increased atmospheric $[\text{CO}_2]$ did not alter photosynthetic rate, but increased average canopy temperature and water use efficiency. Similar effects on entire communities were found by Curtis et al. (1989a).

Some field studies corroborate the temporary stimulation of photosynthetic rate by elevated $[\text{CO}_2]$ found in the laboratory (Woo and Wong 1983; Wulff and Strain 1983; DeLucia et al. 1985; Spencer and Bowes 1986), while others demonstrate a long-term photosynthetic stimulation. Field grown cotton, a C_3 species, exposed to elevated atmospheric $[\text{CO}_2]$ for an entire season maintained rapid growth and high photosynthetic rates throughout the experiment (Radin et al. 1987). Ziska et al. (1990) found the C_3 species Scirpus olneyi maintained higher photosynthetic rates growing in elevated $[\text{CO}_2]$ than ambient $[\text{CO}_2]$ over a two year period in the field.

Bazzaz and Carlson (1984) suggest that C_3 species would be favored as the atmospheric $[\text{CO}_2]$ rises. The increased biomass production and water-use efficiency of C_3 species, relative to C_4 species, could affect the competitive balance and alter community structure (Bazzaz et al. 1985). Similar conclusions for salt marshes were reached by Curtis et al. (1989b). They emphasize the importance of N availability in determining the shifts in community structure and nutrient cycling as larger plants retain more N. The degree of change in community structure caused by elevated atmospheric $[\text{CO}_2]$ s will be determined in part by resource availability in that community (Oechel and Strain 1985).

The differences in response to elevated $[\text{CO}_2]$ vary depending on the species investigated and the experimental design, making it difficult to predict the response of an untested species. It is probable that elevated $[\text{CO}_2]$ will affect growth by changing rates of photosynthesis, photorespiration, and dark respiration and because of variations in species response there will be changes in community structure.

CO_2 FIXATION FROM INTERNAL GAS SPACES OF PLANTS

Plant tissues can be exposed to a 1000-fold range in CO_2/O_2 ratio. Aboveground tissues in agricultural fields experience an atmospheric CO_2/O_2 ratio between 0.0014 - 0.0021 as atmospheric $[\text{CO}_2]$ varies between 300 - 450 $\mu\text{L}\cdot\text{L}^{-1}$ (Brown and Rosenberg 1970; Allen 1971; Verma and Rosenberg 1976). Belowground and internal tissues, however, are exposed to even greater variation as concentrations of O_2 and CO_2 fluctuate according to plant and microbial respiration. In aerobic soils, roots and rhizomes can be exposed to CO_2/O_2 ratios as high as 2.0 due to soil respiration (van Cleemput and Baert 1983), while in flooded soils the rapid decline in O_2 concentration could lead to much higher ratios (Mitsch and Gosselink 1986).

In internal gas spaces the CO_2/O_2 ratio can be 200 times greater than that in the atmosphere ranging between 0.0023 - 0.0300, potentially minimizing photorespiration in

adjacent photosynthetic tissue and serving as a CO₂ source for photosynthesis (Weaver and Wetzel 1980; Longstreth 1989; Chapter 2). Watson and Duffus (1991) demonstrated that the green pericarp of barley could fix CO₂ produced internally, but it accounted for less than 1% of the starch stored in the grain. Fixation of internal CO₂ can play a greater role in maturing cotton fruit and account for 10% of the final dry weight (Wullschleger et al. 1991).

Darkened young legume seed pods can have an internal [CO₂] of 20,000 $\mu\text{L}\cdot\text{L}^{-1}$. This high concentration declines with increasing light intensity suggesting photosynthetic CO₂ fixation (Donkin and Price 1989). The desert shrub Isomeris arborea, bladderpod, can have an internal [CO₂] between 500 - 4,000 $\mu\text{L}\cdot\text{L}^{-1}$ depending on pod age. Carbon fixation of this internal CO₂ can account for 28% of carbon utilized for growth (Goldstein et al. 1991). Eriogonum inflatum, another desert species, has a hollow stem.

Osmond et al. (1987) found these hollow stems contained up to 14,000 $\mu\text{L}\cdot\text{L}^{-1}$ CO₂, but CO₂ uptake rates by the stem from the internal gas space were 6 - 10 times lower than from the atmosphere. They hypothesize that the internal CO₂ pool is potentially more significant for increasing plant water-use efficiency, critical in the desert environment, than plant carbon gain. Therefore CO₂ produced in the tissues of several species can be re-fixed by

photosynthesis, but the importance of this fixation for carbon gain is variable.

Photosynthesis of submerged aquatic species is also influenced by CO₂ accumulation in internal gas spaces. Søndergaard and Wetzel (1980) demonstrated that both accumulation and re-fixation of CO₂ occurred in the internal gas space of the submersed aquatic species Scirpus subterminalis. They estimated that approximately 30% of the CO₂ released by photorespiration was re-fixed within the internal gas space by photosynthesis. Similar results were obtained with Juncus bulbosus L., where CO₂ re-fixed from the internal gas space accounted for 50% of total carbon fixed (Wetzel et al. 1984). Because of the low DIC availability in many aquatic ecosystems the role of the internal CO₂ source in supplying carbon to photosynthesis is likely to assume greater importance in submersed plants.

In the examples above, respiratory CO₂ accumulates within a confined space and is re-fixed by photosynthesis. In general, fixation of CO₂ from internal gas spaces is limited because the gas spaces are relatively small in volume and occur in tissues that are primarily non-photosynthetic. Also, CO₂ accumulates only during the dark period (Setter et al. 1987), therefore, the effect of the increased CO₂/O₂ ratio is transient, being rapidly reduced by photosynthetic activity at dawn.

In contrast, the aerenchyma tissue found in many emergent aquatic plants has a relatively larger storage volume and could provide a continual CO_2 source to the photosynthetic mesophyll through its connection with belowground tissues situated in the CO_2 -rich sediments (Longstreth 1989). Aerenchyma is a porous tissue formed by schizogenous and lysigenous processes in cortex tissue (Esau 1977). The formation of aerenchyma can be induced by ethylene produced in response to low oxygen tensions common in flooded soils (Mitsch and Gosselink 1986; Seliskar 1988). Accumulation of both respiratory CO_2 and CO_2 diffusing into the rhizome gas space from the surrounding sediments could significantly raise the aerenchyma gas space $[\text{CO}_2]$.

Because leaf P_n depends on the intercellular $[\text{CO}_2]$ (C_i), plants with large aerenchyma gas spaces could have a higher C_i due to the concentrated aerenchyma CO_2 source in addition to the atmospheric source (Longstreth 1989). This extra CO_2 source could elevate P_n over that found in C_3 plants lacking aerenchyma, which could effect biomass production.

AERENCHYMA AND ITS ROLE IN GAS TRANSPORT

The role of aerenchyma in relieving anoxia in the rootzone has received considerable attention (Armstrong 1978; Dacey 1980; Curran 1985; Laan et al. 1989).

Transport of O_2 through the aerenchyma to submerged plant organs minimizes anaerobic respiration and increases metabolic energy available for nutrient uptake, carbohydrate mobilization, and root growth. The O_2 supply through the aerenchyma can exceed metabolic demand and excess O_2 can diffuse into the surrounding sediments oxidizing potentially toxic, reduced ions (NH_4^+ ; Mn^{2+} ; Fe^{2+} ; and S^{2-}) (Armstrong 1978; Mitsch and Gosselink 1986; Gries et al. 1990). Oxidation of Fe^{+2} may also facilitate its uptake by plants (Conlin and Crowder 1989).

Oxygen moves through the aerenchyma of the reed, Phragmites australis (Cav.) Trin. ex Steud., to the submerged rhizomes (Armstrong and Armstrong 1988, 1990), although movement is limited in extremely deep water (Weisner 1988). Winter survival of P. australis rhizomes is thought to hinge on downward O_2 transport through dead culms (Brix 1989). Root porosity, a measure of aerenchyma gas space, increased 100% in flooded wheat seedlings in an anoxic growth media and root elongation was maintained by O_2 transport to the root tip, but transport effectiveness declined rapidly at root lengths greater than 100 mm (Erdmann and Wiedenroth 1986; Thomson et al. 1990). Aerenchyma formation appears to be the main determinant of flood-tolerance in species of Rumex. Species growing near the watertable form more aerenchyma than species growing at elevations above the watertable (Laan et al. 1989). Waters

et al. (1989) found that low $[O_2]$ in rice roots at night was rapidly alleviated by a burst of O_2 after sunrise which they attributed to transport of photosynthetically produced oxygen through the aerenchyma tissue. Ability to transport O_2 to root tissues can also influence community zonation patterns by restricting plant growth in deeper water (Yamasaki 1984). A similar phenomenon may partially account for the competitive displacement of T. angustifolia into deeper water by T. latifolia. T. angustifolia survival in deep water may be favored by its larger rhizome which has a greater carbohydrate storage capacity than in T. latifolia (Grace and Wetzel 1981; 1982) and possibly results in a greater rate of O_2 transport to submerged organs.

Oxygen was once thought to move only by diffusion through aerenchyma (Lee et al. 1981; Higuchi 1982). Dacey described a mass flow mechanism driven by a pressure difference in Nuphar and Nelumbo (Dacey 1980; Dacey 1981; Dacey and Klug 1982; Dacey 1987). Subsequently, pressure driven mass flow has been described in the aerenchyma of a variety of plant species (Raskin and Kende 1983; Mevi-Schutz and Grosse 1988a, 1988b; Schröder 1989; Grosse et al. 1991; Armstrong and Armstrong 1991; Hwang and Morris 1991; Armstrong et al. 1992). The downward rate of gas flow can range between 14 and 5,000 $mL \cdot h^{-1}$ (Grosse et al. 1991).

While downward O_2 transport is critical for root survival, upward transport of CO_2 produced by plant and soil respiration to the atmosphere could raise the CO_2/O_2 ratio in leaves. As with downward gas transport, upward transport is possible by diffusion and/or mass flow. In water lilies, the plant can be modeled as a "U", one tip of the "U" being young leaves, the bottom of the "U" being the rhizome located in the sediments and the other tip being older leaves. There is a pressure drop from the young to the old leaves, so that air with 21% O_2 is transported from one aerial tip down into the rhizome and CO_2 -enriched gases are forced up the plant through the other tip of the "U" (Dacey 1981). These conditions permit rhizome gases to be transported quickly to the leaf.

Robe and Griffiths (1988) found that submerged plants of the CAM species Littorella uniflora, grown in CO_2 -enriched media produced greater fresh weight and total surface area than those grown in CO_2 -poor media. CO_2 accumulation from the sediments in the internal gas space of L. uniflora accounts for 70 - 90% of the total CO_2 fixed (Robe and Griffiths 1990). This could be an adaptation to growth in the carbon-poor lakes where the submerged form of this species is found. The terrestrial form of L. uniflora can absorb up to 83% of the total CO_2 fixed from the sediments in spite of the fact that the plant has functional stomata (Nielsen et al. 1991). In the studies

described above the supply of CO_2 to the leaves is improved through the use of an aerenchymatous pathway between the CO_2 -rich sediments and the internal atmosphere of the leaf.

Uptake and utilization of sediment CO_2 by emergent wetland species has also been demonstrated. Photosynthetic uptake of internal CO_2 in Mertensia ciliata could supply CO_2 for growth prior to full leaf development (Billings and Godfrey 1967). Uptake of sediment CO_2 has been shown in Lobelia dortmanna (Wium-Andersen 1971), and Stylites andicola (Keeley et al. 1984). Mature plants of Phragmites australis, a large emergent species, obtain only 1% of the plant carbon requirement from the sediments, however, sediment derived CO_2 may provide a greater percentage of total carbon in young plants (Brix 1990).

Methane (CH_4) is produced in anaerobic soils, but not by plants and therefore it may be used as a tracer to estimate sediment gas transport through plant aerenchyma. Schütz et al. (1989) estimated that as much as 96% of the CH_4 efflux from rice paddy sediments is transported via plant aerenchyma and released to the atmosphere. A study of internal CH_4 concentrations ($[\text{CH}_4]$) in the aerenchyma of several species showed values of up to $5,000 \text{ mg} \cdot \text{m}^{-3}$ and a wide range of efflux rates into the atmosphere (Sebacher et al. 1985). In the same study internal $[\text{CH}_4]$ was $1700 \text{ mg} \cdot \text{m}^{-3}$ and a emission rate was $9.8 \text{ mg} \cdot \text{day}^{-1}$ in cattail. This rate

was approximately two times greater than that estimated by Knapp and Yavitt (1992) for the same species.

The movement of CH_4 through plants suggests that CO_2 could traverse the same pathway and have a profound effect on plant metabolism through its effect on photosynthesis and photorespiration.

PHOTOSYNTHETIC USE OF INTERNAL CO_2 IN TYPHA LATIFOLIA L.

Typha latifolia L. (broadleaf cattail) has the greatest geographical range of the three Typha species found in the United States (Hotchkiss and Dozier 1949; Grace and Harrison 1986). Cattail has many anatomical and physiological characteristics in common with wetland species in addition to a well-developed aerenchyma system. These characteristics are important for determining the importance of aerenchyma gas space [CO_2] in cattail and other wetland species.

Cattail is an herbaceous, rhizomatous perennial and a very efficient producer of biomass (Westlake 1963; McNaughton 1974). Pistillate and staminate flowers are borne on stout vertical stems reaching 3 m in height. Basal leaf sheaths encircle one another near the soil surface forming a compact "stem", and taper into long, linear leaves that can approach 2 m in length and 25 mm in width. Leaves have an extensive central aerenchyma gas space that accounts for up to 50% of the total leaf volume

(Pazourek 1977) and provides a continuous gas pathway between the leaf sheath and tip (Kaul 1974). Rhizomes 0.5 - 3.0 cm in diameter are produced at the leaf base and may extend to 70 cm in length (Grace and Harrison 1986).

The long-term response of T. latifolia to elevated atmospheric $[CO_2]$ is unknown, but cattail is C_3 and P_n should increase with short-term increases in $[CO_2]$ which would occur if the CO_2 in the aerenchyma gas space were available to photosynthetic cells. In order to evaluate the potential photosynthetic use of internal CO_2 by cattail, I have examined several different aspects of cattail anatomy and photosynthetic response.

The initial objective (Chapter 2) was to determine the anatomy of the aerenchyma gas space system and characterize diurnal and seasonal fluctuations of the $[CO_2]$ within the leaf aerenchyma gas space. In cattail, O_2 transport through the aerenchyma to submerged rhizomes is essential for survival (Sale and Wetzel 1983; Dunbabin et al. 1988). Therefore, CO_2 transport to the leaf from the submerged rhizome along the same pathway could supply CO_2 to the photosynthetic process. Several studies have examined fluctuations in aerenchyma $[O_2]$ and $[CO_2]$ in deep water rice (Setter et al. 1987) and Phragmites australis (Brix 1988) and found $[CO_2]$ that could effect carbon gain.

Photosynthetic response to aerenchyma gas space $[CO_2]$ will fluctuate with both PPFD and the aerenchyma gas space

[CO₂]. The general objective of Chapter 3 was to determine if differences in the P_N response to PPFD exist between the adaxial (AD) and abaxial (AB) palisades. The existence of the aerenchyma gas space within the cattail leaf produces a very different leaf structure from that of most terrestrial plants. Terrestrial species usually possess a single photosynthetic palisade that can have different photosynthetic properties when illuminated on the AD or the AB surface (Aston 1978; Raschke et al. 1978). In contrast, the cattail leaf has two photosynthetic palisades, separated by the aerenchyma gas space. The laboratory measurements in Chapter 3 were designed to examine differences between the two palisades in photosynthetic response to PPFD and how these differences influence total P_N.

The objective of Chapter 4 was to examine the response of P_N to [CO₂] in the external atmosphere and in the aerenchyma gas space. The role of [CO₂] in the external atmosphere has been defined for many species (Havelka et al. 1984; Oechel and Strain 1985; Potvin and Strain 1985; Sage et al. 1989). While CO₂ can be assimilated from an internal gas space in some species (see reviews above) the photosynthetic response to changes in [CO₂] in the aerenchyma gas space of cattail is unknown. Cattail may be simultaneously fixing CO₂ from both the atmosphere and the aerenchyma gas space. It is therefore essential to measure

P_N across the epidermis from the atmosphere and across the internal surface from the aerenchyma gas space over a range of $[CO_2]$ to understand the roles of these two CO_2 sources in total leaf fixation of carbon.

Evaluation of the significance of an aerenchyma gas space CO_2 source for cattail will be based on all of the results (Chapters two through four). In Chapter 5, the laboratory results will be interpreted in the context of field measurements to assess the impact of the aerenchyma gas space CO_2 source on cattail growth and reproduction.

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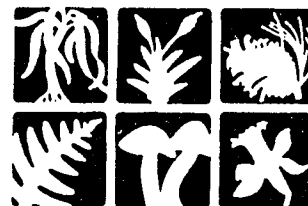
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CHAPTER 2

High Carbon Dioxide Concentrations in Aerenchyma of Typha latifolia L.

INTRODUCTION

The purpose of this study was to determine the diurnal and seasonal patterns of CO₂ concentration ([CO₂]) in leaf gas spaces for the common wetland emergent, Typha latifolia L. (cattail). Anatomical characteristics that provide the basis for unusual leaf [CO₂] were also evaluated. This is part of a larger study that will explore in detail the photosynthetic response of this species in the context of natural variation in leaf [CO₂].

Wetland plants growing in flooded soils generally produce aerenchyma, a tissue that contains extensive gas spaces (Sculthorpe 1985). These gas spaces can serve as a pathway for O₂ movement from leaves to belowground structures and CO₂ movement in the opposite direction (Laing 1940; Dacey 1980; Sculthorpe 1985; Grosse et al. 1991). Transport of CO₂ to the leaves could be very significant for emergent wetland species because increasing [CO₂] above normal atmospheric levels affects the biochemistry of photosynthesis in C₃ plants (the carboxylation reaction increases relative to the oxygenation reaction of ribulose 1,5-bisphosphate

carboxylase/oxygenase, the first enzyme in the C_3 pathway), which can increase the rate of photosynthesis (Ogren 1984; Longstreth 1989; Chapter 1). The effect of high $[CO_2]$, produced experimentally, on photosynthetic rate may vary with species (Spencer and Bowes 1986; Chapter 1). Results from these artificial increases in $[CO_2]$ may not be directly applicable to the natural response of plants in flooded soils but the fact that high $[CO_2]$ produces an increase in the photosynthetic rate and growth rate of many species (Spencer and Bowes 1986), including two C_3 marsh grass species (Rozema et al. 1991), indicates a potentially significant effect of high $[CO_2]$ in intact plants.

Root absorption, transport, and photosynthesis of CO_2 has been described in some plant species. Labeled CO_2 was taken up by Phragmites communis roots and eventually fixed in leaves during a 70-hr experiment in a growth chamber (Brix 1990). Carbon fixation rates of CO_2 absorbed by the roots of a number of submersed plants are very low because of environmental constraints (e.g., Wium-Anderson 1971; Boston et al. 1987). Fixation rates of CO_2 absorbed by roots of Stylites andicola, a fern ally that lacks stomates, are also quite low because of environmental limitations (Keeley et al. 1984). High aerenchyma gas space $[CO_2]$ have been measured in some emergent wetland plants (Laing 1940; Teal and Kanwisher 1966; Brix 1990), but the diurnal and seasonal variation in these

concentrations has not been evaluated. Such temporal variability needs to be determined to begin to estimate the potential impact of high leaf $[CO_2]$ on carbon fixation of emergent wetland plants.

In this study, the $[CO_2]$ in leaf gas spaces was measured at different times during the day and at approximately monthly intervals in cattail plants growing under natural conditions. In addition, anatomical measurements were used to estimate total volume and continuity of the gas-space system in individual plants. These results quantify marked diurnal and seasonal variation in leaf $[CO_2]$. We show here that $[CO_2]$ is substantially higher in cattail leaves than has been reported in leaves of C_3 plants lacking the developed gas-space system found in aerenchyma tissue.

MATERIALS AND METHODS

Plants of Typha latifolia L. (cattail), used in this study, were growing at a site about 1.3 km northeast of the Louisiana State University (LSU) Ben Hur Research Farm in Baton Rouge, Louisiana. At this site, approximately 800 cattail plants are distributed over about 0.05 ha. Other plant species present include Andropogon glomeratus (Walt.), Solidago sempervirens L., Paspalum urvillei Steud., Juncus effusus L. Willd., and Cyperus spp.

Carbon dioxide in leaf gas spaces--Gas samples were collected from leaf sampling "ports" attached between 2 and 5 cm above the sheath of the fifth oldest leaf on each plant sampled at the field site. These leaves containing the ports were oriented at approximately 75 degrees above horizontal. The ports were located approximately 0.5 m above the ground in a canopy that reached a height of 2.0 m in August. Ports were made of 3.8-cm long, 22-gauge syringe needles connected to 15-cm lengths of polyethylene tubing (inside diameter 0.86 mm). Needle tips were inserted into the large gas spaces of the leaves, sealed with vacuum grease and secured with paper tape. The open end of the tubing was sealed with parafilm. The parafilm seal was removed and a 22-gauge syringe needle, attached to a gas-tight syringe (Hamilton Company, Reno, NV), was inserted into the tubing each time gas was sampled. The connection between needle and tubing was sealed with parafilm and 1.0-ml of gas was withdrawn from the leaf interior. After sample withdrawal, the tubing was resealed with parafilm. Air samples were collected from the atmosphere surrounding leaves at the same time as the leaf samples. Syringe needles were immediately sealed by insertion into rubber stoppers and returned to the laboratory. At each sampling time, 6 - 11 leaves on different plants were sampled and these same leaves were sampled throughout the entire day. Different leaves on

different plants were used at each sample date. However, leaves were always of comparable age within a sampling time and across sampling dates.

In the laboratory, syringe contents were injected into nitrogen gas flowing through an infrared gas analyzer (model 225 MK II, Analytical Development Company, Hoddeson, United Kingdom) and the output recorded. For each sample, the area under the curve of the recorder trace was integrated and compared to areas generated by injection of known $[CO_2]$ in air (Matheson Gas Products, East Rutherford, NJ).

Photosynthetic photon flux density (PPFD) was measured perpendicular to and at 1 m above the substrate with a quantum sensor (model LI-190SB, LiCor, Lincoln, NE) at each time $[CO_2]$ was sampled. Air temperature was measured with fine-wire, copper-constantan thermocouples using a microvoltmeter with a built in cold-junction circuit (model HR-33T, WesCor, Logan, UT).

Evaluation of the gas pathway--The continuity of gas spaces between rhizomes and leaves was evaluated by dye movement. Plant sections containing rhizome-shoot transitions were prepared by excising leaves and rhizomes approximately 5 cm from the center of the transition. Evan's blue dye (0.4% w/v) was applied to the surface from which leaves had been excised, a plastic tube was tightly fitted over this cut surface, and parafilm was wrapped

around the junction between the tissue and tube. The rhizome-shoot transition was immersed in water and a slight air pressure (0.03 MPa) was applied to drive dye into the tissue. Dye movement in rhizomes was also measured in an identical manner using 5-cm segments of rhizomes, cut 5 cm distal to a rhizome-shoot transition.

Leaf gas-space volume was estimated from measurements of the cross-sectional area of leaves occupied by gas spaces. The gas-space area in each leaf cross section was quantified from photographic enlargements using a digitizer interfaced to a microcomputer (Apple II+, Apple Corporation, Cupertino, CA). Sections were taken at 15-cm intervals along leaves, and total enlargements of sections were between X89 and X110.

Rhizome gas-space volume was estimated in six separate rhizomes. Three samples were dissected from the layer between the epidermis and vascular stele (where gas spaces occurred) from each of three each rhizomes (Fig. 2.3C). Using a pycnometer, porosity was estimated from measurements of the volume displaced by samples before and after crushing to remove gas spaces (Burdick 1989). Based on field measurements, we assumed that each shoot was attached to two rhizomes and that the average length of rhizome connected to each shoot was 17 cm. The product of the total rhizome volume containing aerenchyma and the

measured porosity was taken as an estimate of rhizome gas-space volume per plant.

RESULTS AND DISCUSSION

Field measurements of leaf $[CO_2]$ --The maximum leaf $[CO_2]$ was well above atmospheric $[CO_2]$ at each sampling date (Fig. 2.1). The maximum mean leaf $[CO_2]$ at any sampling time varied during the season and was $2,551 \mu L \cdot L^{-1}$ on 28 May, $6,316 \mu L \cdot L^{-1}$ on 28 August, and $2,414 \mu L \cdot L^{-1}$ on 23 October. Atmospheric $[CO_2]$ adjacent to leaves was also generally higher at dawn than at noon, but the absolute amplitude of this variation was much smaller than for leaf $[CO_2]$ (Fig. 2.1).

At each sampling date leaf $[CO_2]$ changed dramatically during the day, with generally the highest values being found at dawn. As the day progressed, leaf $[CO_2]$ declined (the lowest values were reached between 1300 and 1500 hr) and then increased again later in the day. Leaf $[CO_2]$ exceeded atmospheric $[CO_2]$ for about $4 \text{ hr} \cdot \text{d}^{-1}$ in early July and for approximately $12 \text{ hr} \cdot \text{d}^{-1}$ in late September (Fig. 2.1). The lowest leaf $[CO_2]$ values were generally equal to or greater than the atmospheric values at midday when photosynthetic rates should have been at a maximum. In contrast, a previous study has shown that the leaf $[CO_2]$ in eight C_3 species that lack large gas spaces was 65 to

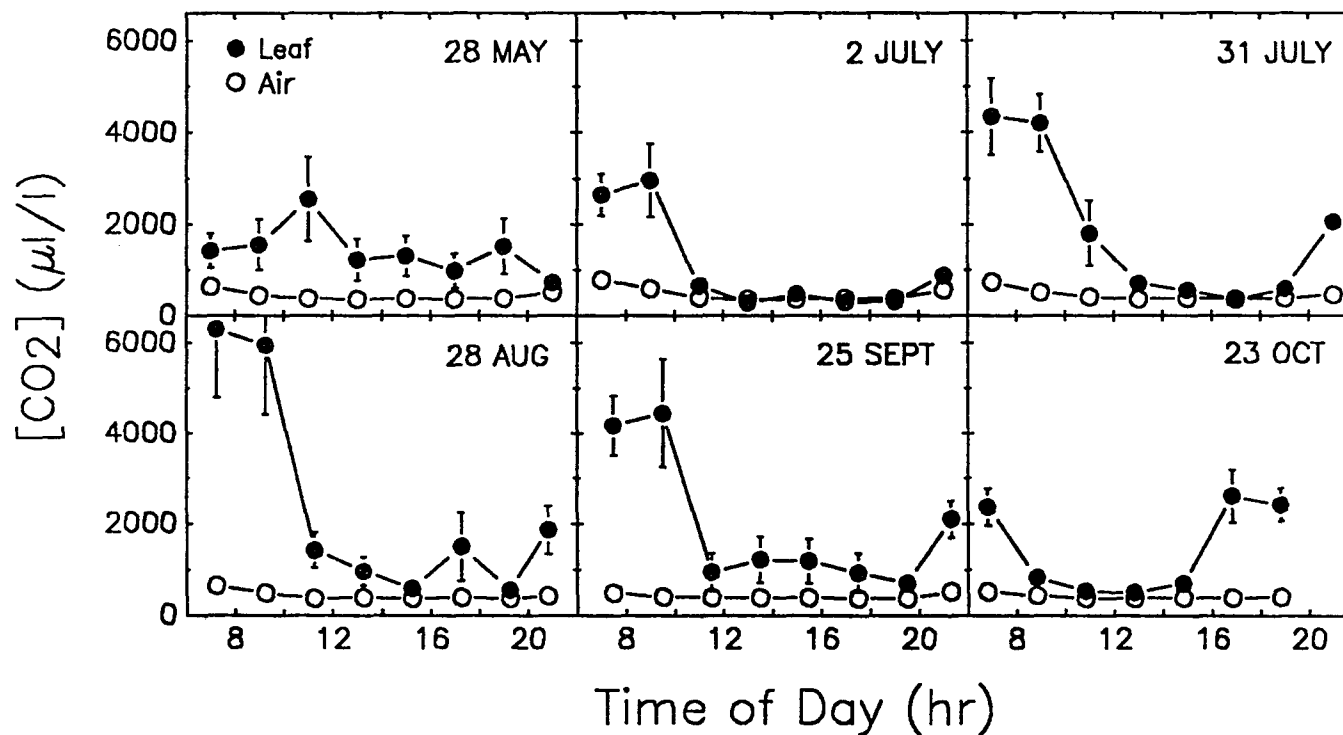


Figure 2.1. Diurnal measurements of $[CO_2]$ from leaf gas spaces of *Typha latifolia* L. (closed circles) and atmosphere outside the leaves (open circles) at six different times during the growing season. Values are means \pm 1 SE where $N = 6$ to 11.

$115 \mu\text{L}\cdot\text{L}^{-1}$ below an ambient $[\text{CO}_2]$ of $305 \mu\text{L}\cdot\text{L}^{-1}$ (Wong et al. 1985). In a laboratory study of Phragmites australis, Brix (1988) reported that $[\text{CO}_2]$ in shoot gas spaces was always above atmospheric $[\text{CO}_2]$ during the light period. In the inflated, photosynthetic stem of Eriogonum inflatum, the minimum $[\text{CO}_2]$ was over ten times atmospheric $[\text{CO}_2]$ during the day although there appeared to be a diffusion barrier between this CO_2 pool and photosynthetic cells (Osmond et al. 1987).

PPFD and air temperature, measured concurrently with $[\text{CO}_2]$, showed typical diurnal patterns at each sampling date (Fig. 2.2). While there were brief periods of cloudiness during a few of the sampling dates, cloud cover only became a significant factor on the afternoon of 23 October. These environmental patterns suggest that the diurnal changes in leaf $[\text{CO}_2]$ (Fig. 2.1) may be a function of photosynthetic rate. That is, leaf $[\text{CO}_2]$ is highest when photosynthetic demand for CO_2 is relatively small (early and late in the photoperiod) and leaf $[\text{CO}_2]$ is lowest when photosynthetic demand for CO_2 is relatively large (from 1000 hr to 1650 hr; Fig. 2.2). Although the range was much smaller than shown here, a similar qualitative pattern for maximum and minimum leaf $[\text{CO}_2]$ was reported for laboratory grown Phragmites australis (Brix 1988). In contrast, the diurnal pattern for methane

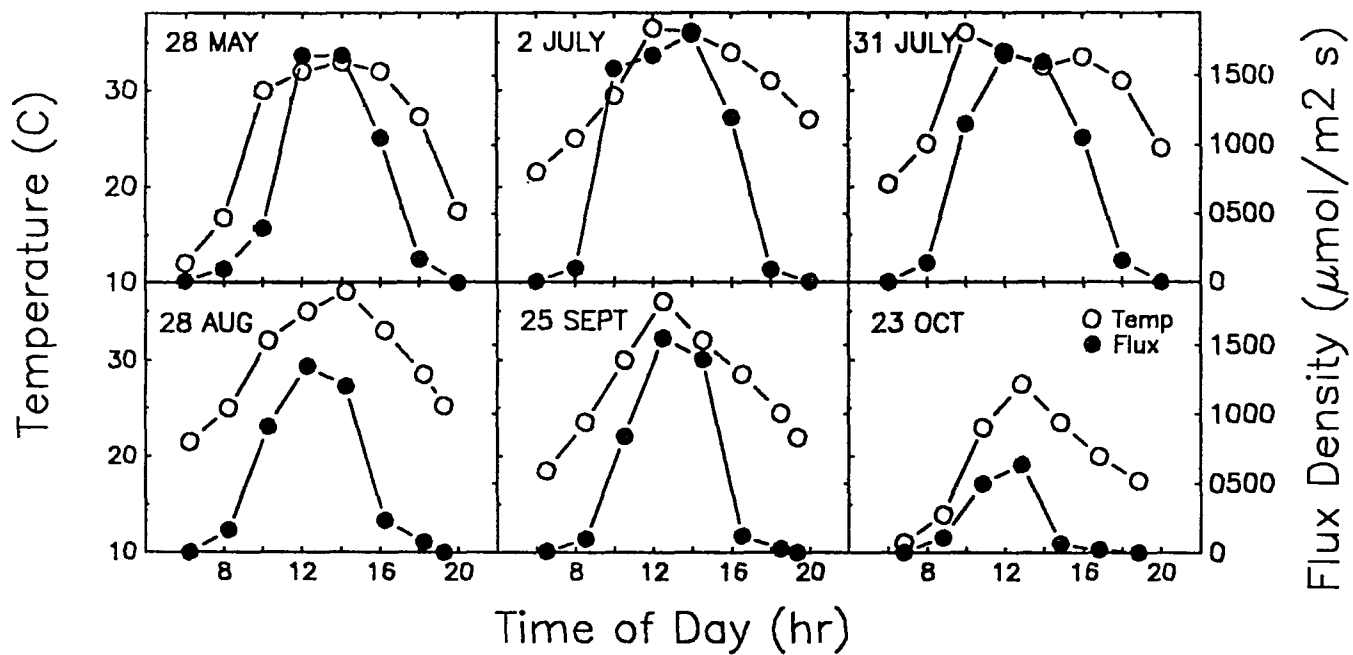


Figure 2.2. Air temperature (open circles) and PPFD (closed circles) measured at six different times during the growing season.

release from rice is apparently correlated with soil temperature and therefore soil microbial activity (Schütz et al. 1989). Presumably the methane release pattern is opposite the pattern for maximum and minimum leaf $[CO_2]$ in cattail (Fig. 2.1) because methane is not appreciably metabolized by plants.

Elevated leaf $[CO_2]$ (Fig. 2.1) is probably derived from 1) CO_2 generated by microbial activity in the soil sediments, and 2) plant respiratory CO_2 diffusing into the aerenchyma system. Our measurements were not designed to differentiate between these two sources of CO_2 , but there is considerable precedence that gases generated in flooded soils are transported up plants through aerenchyma systems (e.g., Dacey 1980; Mevi-Schutz and Grosse 1988; Grosse et al. 1991). Sebacher et al. (1985) reported that methane and tracer gases move from the root zone of several wetland species, including cattail, to the atmosphere surrounding leaves. While the belowground gas-space volume is estimated to be quite small here (about 6% of the total gas-space volume), the fact that a mass flow system for internal transport occurs in some wetland species indicates that the high $[CO_2]$ generated by soil microbes could be the major factor controlling leaf $[CO_2]$. Sebacher et al. (1985) report pressures up to 100 Pa in cattail leaves and mass flow of methane through plants. We have found methane

in leaf gas spaces (Appendix A), and have found pressures up to 120 Pa (Appendix B).

Aerenchyma characteristics--Gas spaces occurred in all parts of the plant, and the largest spaces were in leaves (Fig. 2.3A). From sections, it was apparent that gas-space tissue extended into the rhizome-shoot transition (Fig. 2.3B). Under slight pressurization, dye moved readily through the rhizome-shoot transition via a porous, outer layer and through a similar layer in the rhizome (Fig. 2.3C). Vascular bundles appeared concentrated in the stele but were also present in the outer layer where the dye moved. The dyed area occupied $68\% \pm 2\%$ of the total area in 40 cross sections of rhizomes taken from 25 different plants. In both rhizomes and rhizome-shoot transitions, application of low pressure produced vigorous bubbling in submerged portions of the tissue. Aerenchyma gas-space volume ranged between 5 cm^3 in youngest leaves to 30 cm^3 in old leaves (Appendix C). Gas-space volume expressed per unit leaf surface area ranged from $0.14 \text{ cm}^3 \cdot \text{cm}^{-2}$ in older leaves to $0.05 \text{ cm}^3 \cdot \text{cm}^{-2}$ in younger leaves. This is approximately seven to 16 times the volume per unit leaf area found in Alternanthera philoxeroides (Longstreth et al. 1985) and Gossypium hirsutum (J. Smith and D. Longstreth, unpublished data), two C_3 dicot species that lack large and continuous gas spaces.

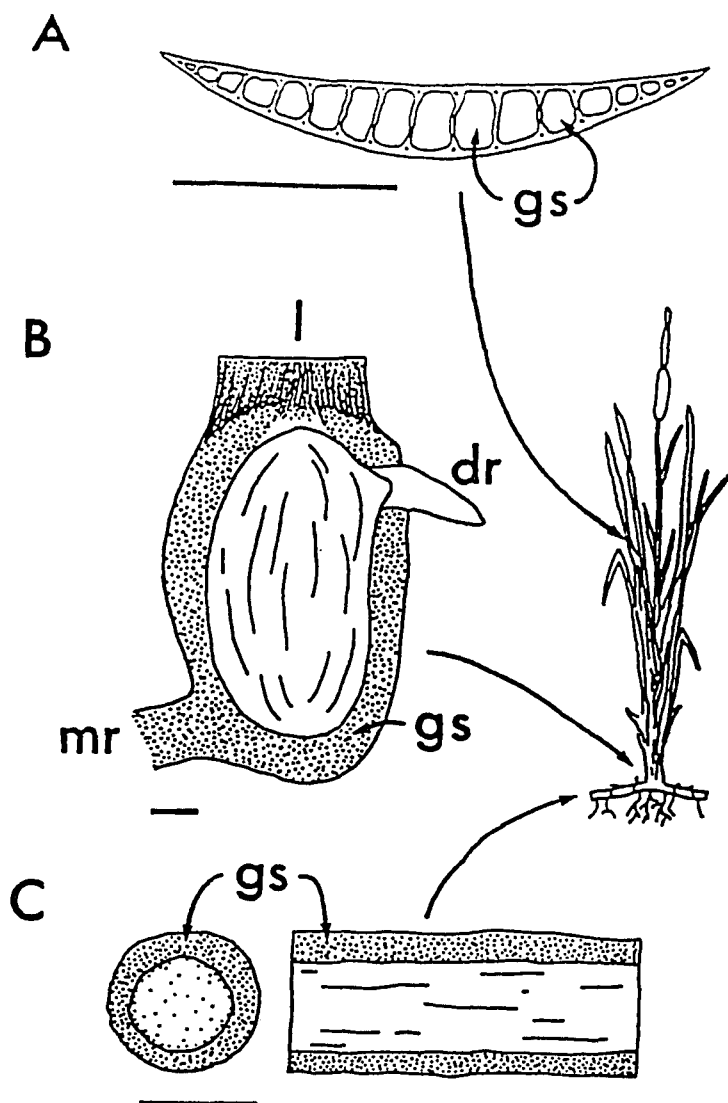


Figure 2.3. Leaf cross section (A.), rhizome-shoot transition (B.), and cross and longitudinal sections of rhizomes (C.) of *Typha latifolia* L. Location of gas spaces, gs; leaf base, l; developing rhizome, dr; mature rhizome, mr. Horizontal bars = 1 cm in each section.

Shoots in our population generally possessed 12 leaves. The total gas-space volume of a shoot with 12 leaves was $241 \pm 13 \text{ cm}^3$. We estimated the aerenchyma volume in rhizomes associated with a shoot to be 13.8 cm^3 or about 6% of the aerenchyma volume in the aboveground part of the plant.

Possible consequences--In most terrestrial systems, microbial degradation of organic matter and plant respiration of sugars produces CO_2 that is returned to the atmosphere where ambient $[\text{CO}_2]$ can be limiting to photosynthesis. In emergent C_3 plants like cattail, however, $[\text{CO}_2]$ is elevated in internal gas spaces and this high $[\text{CO}_2]$ will favor carboxylation over oxygenation of ribulose 1,5-bisphosphate (Ogren 1984). The potential result is that during periods of elevated internal $[\text{CO}_2]$, there will be an increase in carbohydrate formation and growth. High aerenchyma gas space $[\text{CO}_2]$ may play a significant role in maintaining productivity in wetlands that are dominated by plants with aerenchyma.

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CHAPTER 3

Leaf Structure and Photosynthetic Response of Adaxial and Abaxial Leaf Surfaces of Typha latifolia L. at Different Photosynthetic Photon Flux Densities

INTRODUCTION

The anatomy of a "typical" C_3 leaf can be described as an asymmetrical structure in which a single layer of photosynthetic mesophyll is located between an adaxial (AD) epidermis and an abaxial (AB) epidermis (Esau 1977). The mesophyll is generally composed of two cell types, a palisade parenchyma adjacent to the adaxial epidermis and immediately below a spongy parenchyma adjacent to the abaxial epidermis. In cattail (Typha latifolia L.) the leaf structure is quite different from that described above. Unlike the "typical" C_3 leaf, a cattail leaf has two separate photosynthetic mesophylls separated by large gas spaces that are part of the aerenchyma tissue system that connects the gas spaces of above and belowground portions of the plant (Chapter 2; Pazourek 1977). Leaf gas spaces are composed of long channels parallel to the long axis of the leaf. These channels in mature leaves are divided by porous horizontal partitions (Kaul 1974).

The single photosynthetic layer (palisade and spongy parenchyma) of the "typical" C_3 leaf can be illuminated on

either the AD or AB epidermis to produce positive net photosynthetic CO_2 uptake rate (P_n), although the gas exchange rates across the two epidermis' are rarely identical (Aston 1978; Raschke et al. 1978). When oriented perpendicular to the solar beam the densely packed palisade parenchyma of the "typical" C_3 leaf shades the spongy parenchyma below. As both parenchyma layers can have similar photosynthetic capacities, some reduction in P_n could result from this internal shading (Outlaw et al. 1976). Shading can cause the spongy parenchyma to have biochemical characteristics similar to shade leaves, while the palisade parenchyma has characteristics similar to sun leaves (Terashima and Inoue 1984, 1985). These biochemical differences between the palisade and spongy cells are reflected as differences in photosynthetic response to light (Terashima 1986). In cattail, shading is more extreme because the aerenchyma gas space, which is several times the thickness of the palisade layer, separates the two palisades. Each palisade has an external surface covered by a normal layer of epidermal cells punctuated by stomata and an unusual internal surface covered by a layer of cells facing the aerenchyma gas space.

A primary goal of this study was to determine the relative contribution of illuminated and shaded palisades of intact leaves to P_n measured at different PPFDs. A second goal was to determine if there were absolute

differences in P_n response to PPFD of the AD and AB palisades. A third goal was to evaluate the anatomical structure of the cattail leaf and characterize the cells that form the boundary between the palisade and the aerenchyma gas space. Cattail gas exchange response to PPFD and anatomical characteristics of the palisade are important for determining if the palisade could acquire CO_2 for photosynthesis from the concentrated CO_2 source found in the aerenchyma gas space (Chapter 2).

MATERIALS AND METHODS

Plant Material--Cattail plants used in this study were collected at a site 1.3 km northeast of the Louisiana State University (LSU) Ben Hur Research Farm in Baton Rouge, Louisiana. Following collection, roots were washed of soil and potted in moist vermiculite in 20 cm diameter plastic pots. Each pot was placed in a 23-cm diameter, plastic tub with 1.5 L of deionized water and fertilized with approximately one gram of 14-14-14 Osmocote fertilizer (Sierra Chemical Company, Milpitas, CA). All plants were grown in a greenhouse under ambient conditions of PPFD and temperature. Two weeks prior to all experiments, plants were transferred to a growth chamber with a 13 h photoperiod and day/night temperatures of 30/22 C. Growth PPFD was approximately $300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the leaf tip and $180 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 80 cm above the leaf base where all gas

exchange measurements were performed. Plants were watered with 0.5 strength Hoaglands solution (Epstein 1972) every third day and water depth was maintained at approximately 2 - 4 cm below the surface of the vermiculite.

Gas Exchange Measurements--Rates of CO₂ and H₂O vapor exchange were measured in a clamp-on cuvette with an open gas exchange system. One-sided leaf area for gas exchange measurements of total intact leaves ranged between 4.1 - 5.2 cm². A leaf area of 3.25 cm² was used for measurements of individual leaf palisades (either AD or AB). Leaf temperature was 20 ± 2 C as measured with fine-wire copper-constantan thermocouples. PPFD was provided by a carousel projector with a 300 W bulb (model Ektagaphic III E, Eastman Kodak Corporation, Rochester, NY) and measured at the leaf surface with a quantum sensor (model 190-SB, LiCor, Lincoln NE). Different PPFDs were obtained by varying the distance between the projector and the leaf surface or adding filters (exposed photographic film). The CO₂ concentration ([CO₂]) in the gas stream for all experiments was 350 ± 5 μL·L⁻¹, obtained by mixing 1% CO₂ with CO₂-free air using proportional gas controllers (model 8141, Matheson Gas Products, East Rutherford, NJ). The gas stream was then humidified by bubbling it through acidified water and brought to a dew point between 14.6 - 15.2 C in a stainless steel condenser placed in a temperature-controlled water bath. The gas stream entered the cuvette

on both the AD and AB surfaces of the leaf and mixed freely during total leaf measurements. Gas exchange measurements from individual AD and AB surfaces were obtained by adding two plexiglass plates that effectively split the cuvette into two separate chambers for each leaf surface and kept the two gas streams separate. A mass flow meter (model 8142, Matheson Gas Products, East Rutherford, NJ) and a water manometer were placed in each gas stream to produce similar flow rates and air pressures over the AD and AB surfaces. Gas streams from the leaf surfaces were routed to the differential infrared gas analyzer (model 225 MK II, Analytical Development Company, Hoddeson, United Kingdom) and dew point hygrometer (model 911, EG & G Corporation, Waltham, MA) for measurement or were vented to the room (Fig. 3.1). Absolute $[CO_2]$ was measured with an infrared gas analyzer (model LCA-2, Analytical Development Company, Hoddeson, United Kingdom). All gas lines carrying humidified air were stainless steel or teflon tubing. The infrared gas analyzers, thermocouples, mass flow meters, and dew point hygrometer were connected by a data handling system (model 91A, Cyborg Corporation, Newton, MA) to a microcomputer (model II+, Apple Computer Corporation, Cupertino, CA). Real time exchange rates of CO_2 and H_2O vapor were calculated after von Caemmerer and Farquhar (1981).

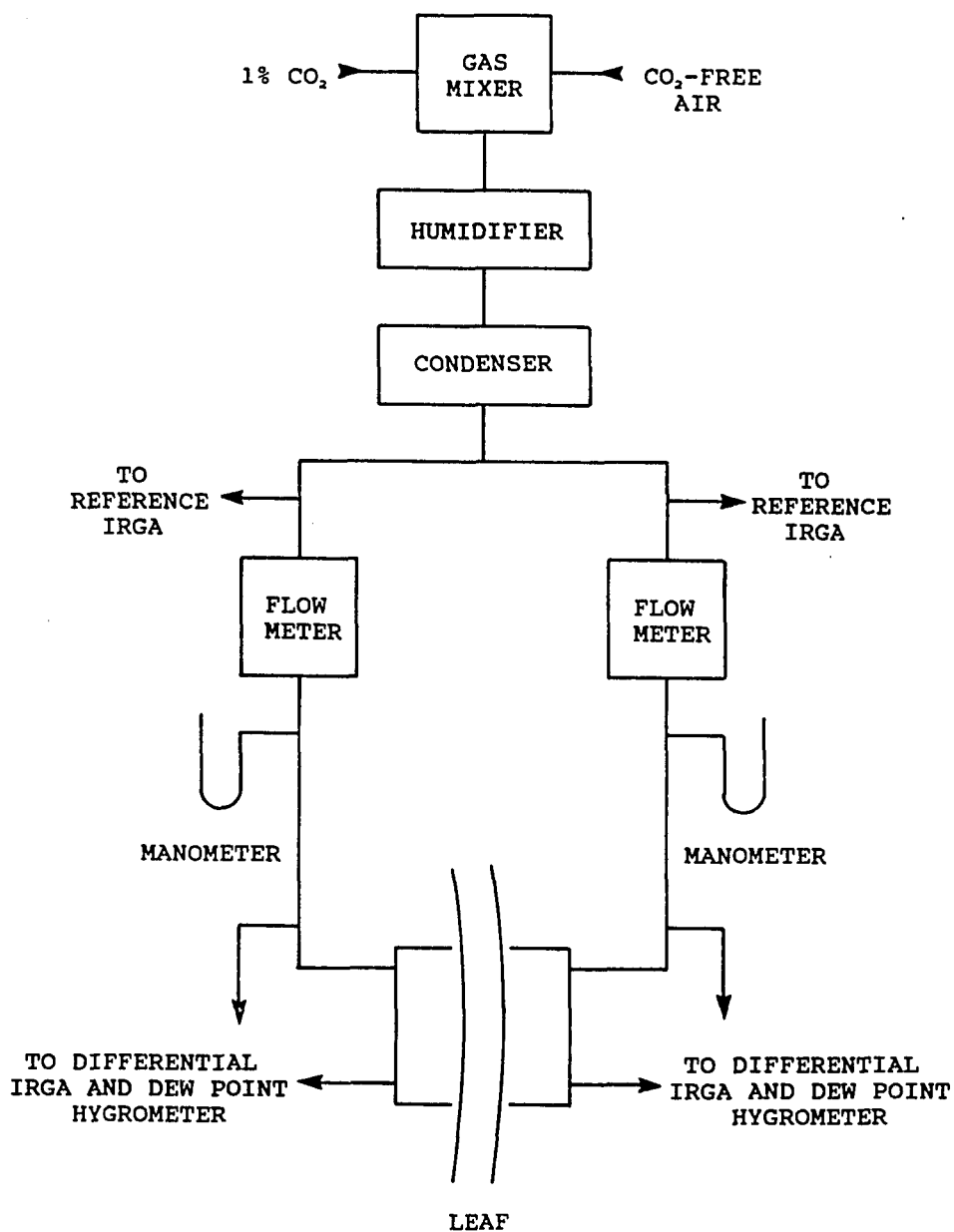


Figure 3.1. General schematic of the gas exchange system showing gas flow arrangement for the split-chamber cuvette.

The gas exchange system measures exchange between the leaf and the surrounding external atmosphere. In intact cattail leaves, however, there may also be gas exchange between the palisade and the aerenchyma gas space. The aerenchyma gas space $[CO_2]$ in laboratory plants was lower than that found in the field (Chapter 2) averaging approximately $500 \mu L \cdot L^{-1}$. Cattail leaves were partially dissected to produce a portion of the leaf with only a single palisade (either AD or AB) bounded on one side by epidermis (henceforth the epidermal surface) and on the other side by a layer of non-chlorophyllous cells that before dissection faced the aerenchyma gas space (henceforth the internal surface). The dissected portion of the leaf was placed in the split-chamber cuvette to measure P_n across the epidermal surface at different PPFDs, while the $[CO_2]$ adjacent to the internal surface was kept constant at approximately $350 \mu L \cdot L^{-1}$.

The effect of dissection on leaf water balance was measured on paired leaves with a pressure bomb. After 3 - 4 h of gas exchange, dissected leaves had a Ψ_w that was less than 0.1 MPa ($n = 7$) below that of control leaves. Also, transpiration from the dissected portion of the leaf was constant for the 3 - 4 h period, indicating that water supply to the palisade had not been substantially disrupted.

Microscopy and PPFD Transmittance Measurements--For light microscopy entire leaf cross sections, approximately 3 mm x 4 mm x 15 mm, were fixed in formalin-acetic acid-alcohol (5% formalin, 5% acetic acid, 45% ethanol, 45% distilled water) and dehydrated in a graded ethanol series. The cross sections were embedded in paraffin and sectioned at 12 μ m on a rotary microtome, stained with toluidine blue and photographed. For scanning electron microscopy (SEM), AD and AB leaf palisades, approximately 4 mm x 5 mm x 3 mm, were fixed for 2 h in 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2), rinsed in distilled water and dehydrated in a graded ethanol series. After critical point drying, sections were mounted on stubs, sputter coated with approximately 200 Å of gold-palladium, and viewed with a SEM (model S-260, Cambridge Instruments, Cambridge UK). Stomatal densities were calculated on AD and AB epidermal surfaces from six mature leaves. Mean stomatal density was estimated for each surface from stomatal counts in each of 10 randomly placed 0.09 mm² quadrats on an X75 SEM image. Chlorophyll concentrations were determined in 80% (v/v) acetone after Arnon (1949).

To examine PPFD transmittance through the cattail palisade, ten-cm long sections from mature leaves were excised approximately 80 cm above the base of growth chamber plants. These sections were taped against a 5 mm thick piece of clear plexiglass and oriented perpendicular

to PPFD provided by a carousel projector (model Ektagaphic III E, Eastman Kodak Corporation, Rochester, NY). PPFD at the leaf surface was $1428 \pm 1.0 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (mean \pm se; $n = 8$) and PPFD at different distances through the leaf section was measured using a photodiode (Hamamatsu Corporation, Bridgewater, NJ) calibrated against a quantum sensor (model LI-190SB, LiCor, Lincoln, NE). PPFD transmittance through the leaf section was determined by measuring PPFD passing through the intact leaf, through the illuminated palisade with the shaded palisade removed, but all gas partitions intact, and through the illuminated palisade with both the shaded palisade and all gas partitions removed (see Fig. 3.7 for locations).

RESULTS

Gas Exchange Measurements-- P_n of intact cattail leaves was saturated at a PPFD of approximately $900 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when illuminated on the AD palisade and approximately $700 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when illuminated on the AB palisade (Fig. 3.2A). Maximum P_n of the total intact leaf was approximately $6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when the AD palisade was illuminated, and $4 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ when the AB palisade was illuminated. Differences in conductance between the AD and AB palisades corresponded to the differences in P_n . Conductance of the AB palisade was 66% that of the AD at

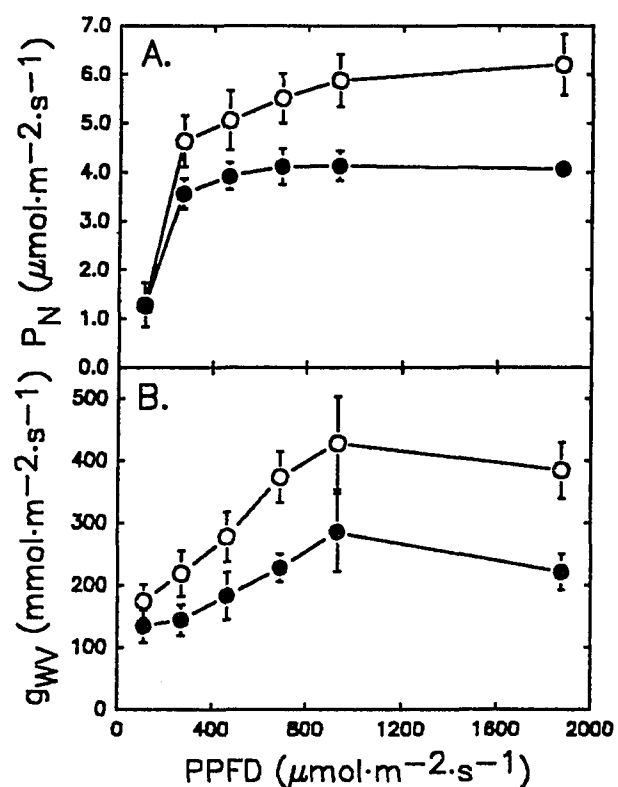


Figure 3.2. P_N (A.) and conductance (B.) at different PPFDs for *Typha latifolia* L. leaves illuminated on the adaxial epidermis (open symbols) or abaxial epidermis (closed symbols). The $[\text{CO}_2]$ was approximately $350 \mu\text{L}\cdot\text{L}^{-1}$. Values are means \pm SE ($n=5-6$).

930 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPFD and a similar difference was observed at other PPFDs (Fig. 3.2B).

Measurements of intact leaves with the split-chamber cuvette permitted separate determinations of P_N for the illuminated and shaded palisades. When leaves were illuminated from the AD side, P_N of the AD palisade reached a maximum of 6.2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at a PPFD of 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, while the maximum P_N of the AB palisade reached a maximum of 1.37 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 3.3A). Conductance to water vapor was highest at the maximum PPFD, with values for the shaded AB palisade ranging between 56% and 76% of those for the illuminated AD palisade (Fig. 3.3B). A similar response was seen when leaves were illuminated from the AB side (Figs. 3.3C and D). The maximum P_N for the illuminated AB palisade was 3.8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 3.3C), 61% the rate of the illuminated AD palisade (Fig. 3.3A). The AB palisade saturated at approximately 500 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 3.3C). Again P_N of the shaded AD palisade increased as PPFD on the illuminated AB palisade increased. Conductance for the shaded AD palisade was 12% - 18% that for the illuminated AB palisade (Fig. 3.3D). Reversing the leaf orientation in the split-chamber cuvette produced the same differences between the AD and AB palisades.

P_N for just the epidermal surface of the AD palisade was determined with a known $[\text{CO}_2]$ adjacent to the internal

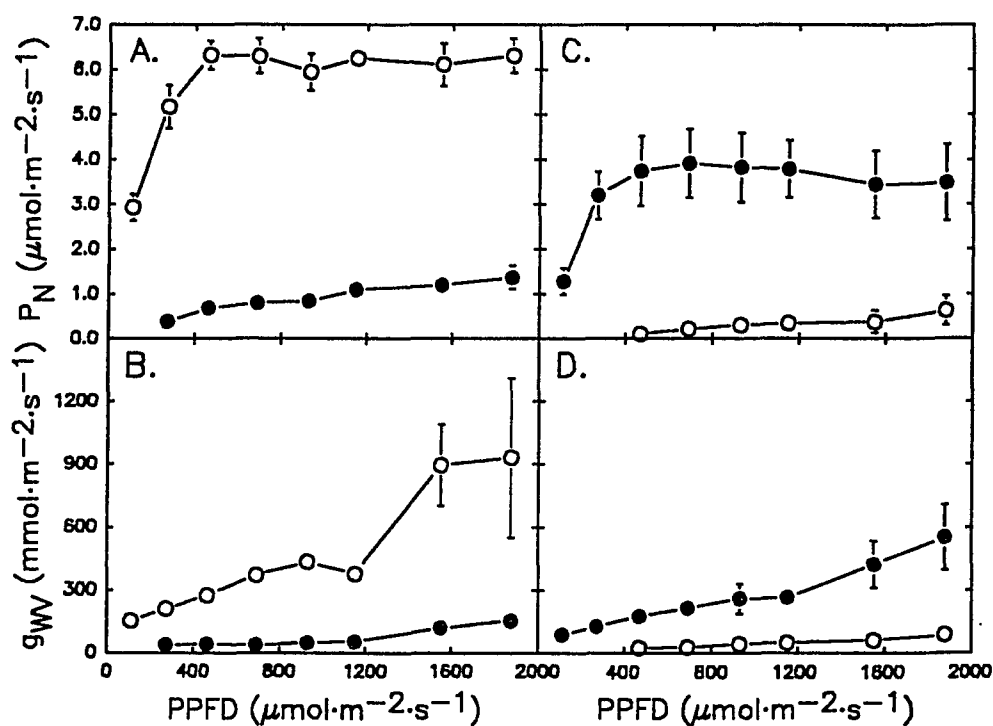


Figure 3.3. P_N (A. and C.) and conductance (B. and D.) of isolated adaxial palisade (open symbols) and abaxial palisade (closed symbols) at different PPFDs for *Typha latifolia* L. leaves in the split-chamber cuvette. A and B. Adaxial illumination. C and D. Abaxial illumination. The $[\text{CO}_2]$ was approximately $350 \mu\text{L}\cdot\text{L}^{-1}$. Values are means \pm SE (n = 5 - 7).

cell surface which was exposed by removal of the AB palisade. P_N across the epidermis of the AD palisade of dissected leaves was PPFD saturated at approximately $600 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Fig. 3.4A) and the maximum P_N was $5.5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Conductance appeared to increase as PPFD increased, although conductances measured at PPFDs above saturation were quite variable (Fig. 3.4B).

Microscopy and PPFD Transmittance Measurements--The leaf AD and AB palisades were separated by a series of large parallel internal gas spaces that run the long axis of the leaf and form part of the aerenchyma tissue system (Fig. 3.5A). In cross-section, the AD and AB palisades were visually similar. On both surfaces the palisade is located in defined semi-circular regions separated by vascular bundles (Figs. 3.5A and B). An apparent spongy mesophyll was lacking. The chlorophyll concentrations for the two palisades were not significantly different using a t-test ($\text{AD} = 41.8 \pm 1.9 \mu\text{g}\cdot\text{cm}^{-2}$; $\text{AB} = 48.7 \pm 1.4 \mu\text{g}\cdot\text{cm}^{-2}$; values are means \pm SE, $n = 8$; $p = 0.013$).

The internal surface of the palisade which faced the aerenchyma gas space was covered by 3 - 4 layers of non-chlorophyllous cells that separated the palisade from the aerenchyma gas space (Figs. 3.5A and B). The cells appeared to be tightly connected together with no apparent openings to facilitate gas transfer between the palisade and the aerenchyma gas space. At the center of a gas

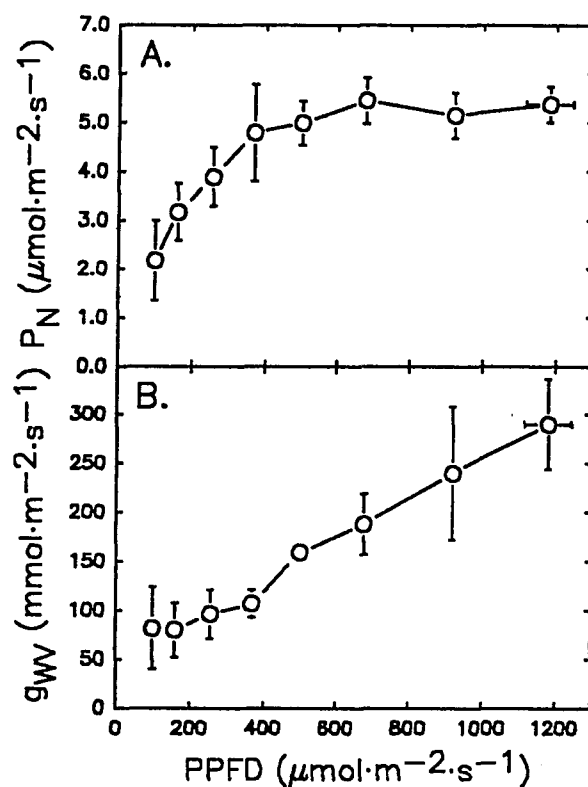
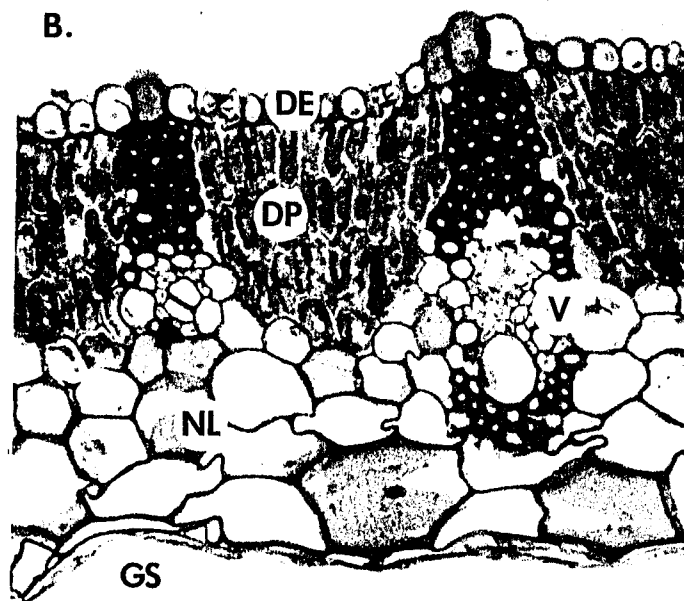
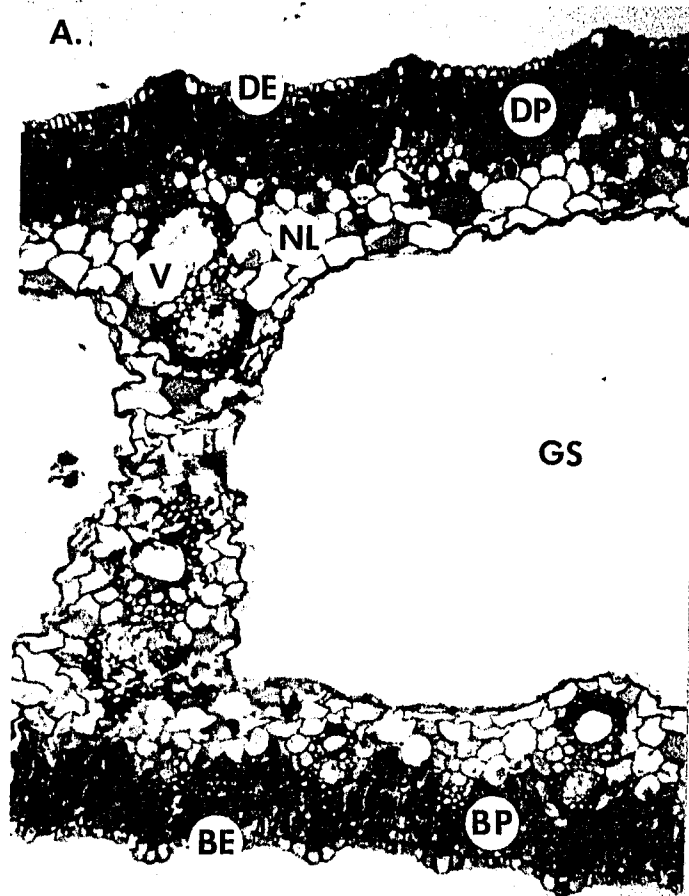


Figure 3.4. P_N (A.) and conductance (B.) at different PPFDs of the adaxial palisade for dissected *Typha latifolia* L. leaves. Leaves were illuminated on the AD surface and the $[\text{CO}_2]$ on the epidermal and internal surfaces was approximately $350 \mu\text{L}\cdot\text{L}^{-1}$. Values are means \pm SE ($n = 4$).

Figure 3.5. Light micrographs of Typha latifolia L. leaf cross-sections in paraffin. A. Light micrograph (X100). B. Light micrograph (X400). Legend: DE, adaxial epidermis; BE, abaxial epidermis; DP, adaxial palisade; BP, abaxial palisade; V, vascular bundle; NL, non-chlorophyllous cell layer; GS, aerenchyma gas space.



channel the depth of the non-chlorophyllous cell layer was $88 \pm 6 \mu\text{m}$ (mean \pm SE, $n = 10$) and $102 \pm 4 \mu\text{m}$ (mean \pm SE, $n = 10$) for AD and AB palisades, respectively (Fig. 3.5A). The depths of the AD and AB palisades were similar at $75 \pm 3 \mu\text{m}$ (mean \pm SE, $n = 10$) and $83 \pm 2 \mu\text{m}$ (mean \pm SE, $n = 10$), respectively (Fig. 3.5A).

The epidermal surface of the palisade was covered with ridges parallel to the long axis of the leaf indicating locations of the underlying vascular bundles (Fig. 3.6). Stomata were not found on the epidermal ridges. Guard cells appeared more similar to dicot guard cells which are "kidney-shaped", than monocot guard cells which are "dumbbell-shaped" (Fig. 3.6). Stomatal density on the AD epidermis was $620 \pm 116 \text{ mm}^{-2}$, approximately 12% greater than the $550 \pm 121 \text{ mm}^{-2}$ found on the AB epidermis, but was not statistically significant using a t-test ($p = 0.035$). SEM images of the internal surface showed it undulated over the palisade and no regular gas openings were apparent (Fig. 3.7).

Approximately $161 \pm 2 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ (mean \pm se; $n = 8$) of the PPFD incident on the AD epidermis ($1428 \pm 1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) was transmitted through the AD palisade, and $133 \pm 3 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ reached the internal surface of the AB palisade from the illuminated AD palisade (Values summarized as percentages on Fig. 3.8). Only $21 \pm 1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ of PPFD incident on the AD epidermis was transmitted through the

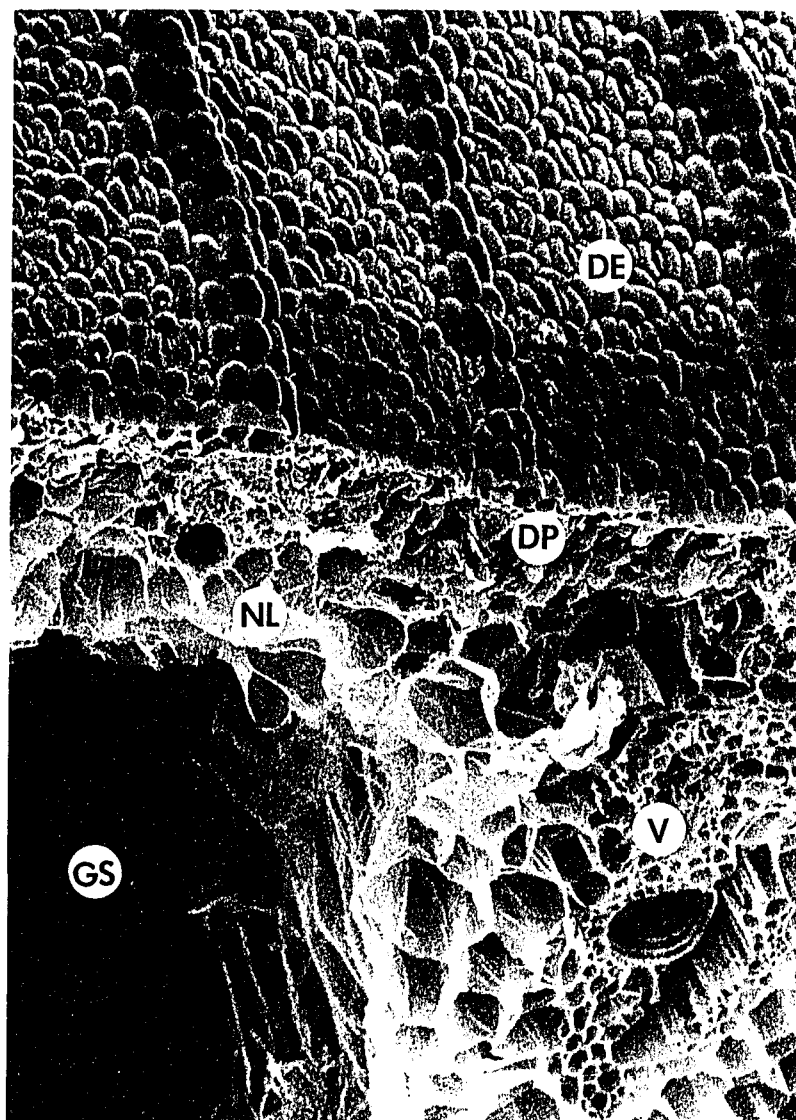


Figure 3.6. Scanning electron micrograph of *Typha latifolia* L. leaf cross-section showing AD epidermis (X250). Legend: DE, adaxial epidermis; DP, adaxial palisade; V, vascular bundle; NL, non-chlorophyllous cell layer; GS, aerenchyma gas space.

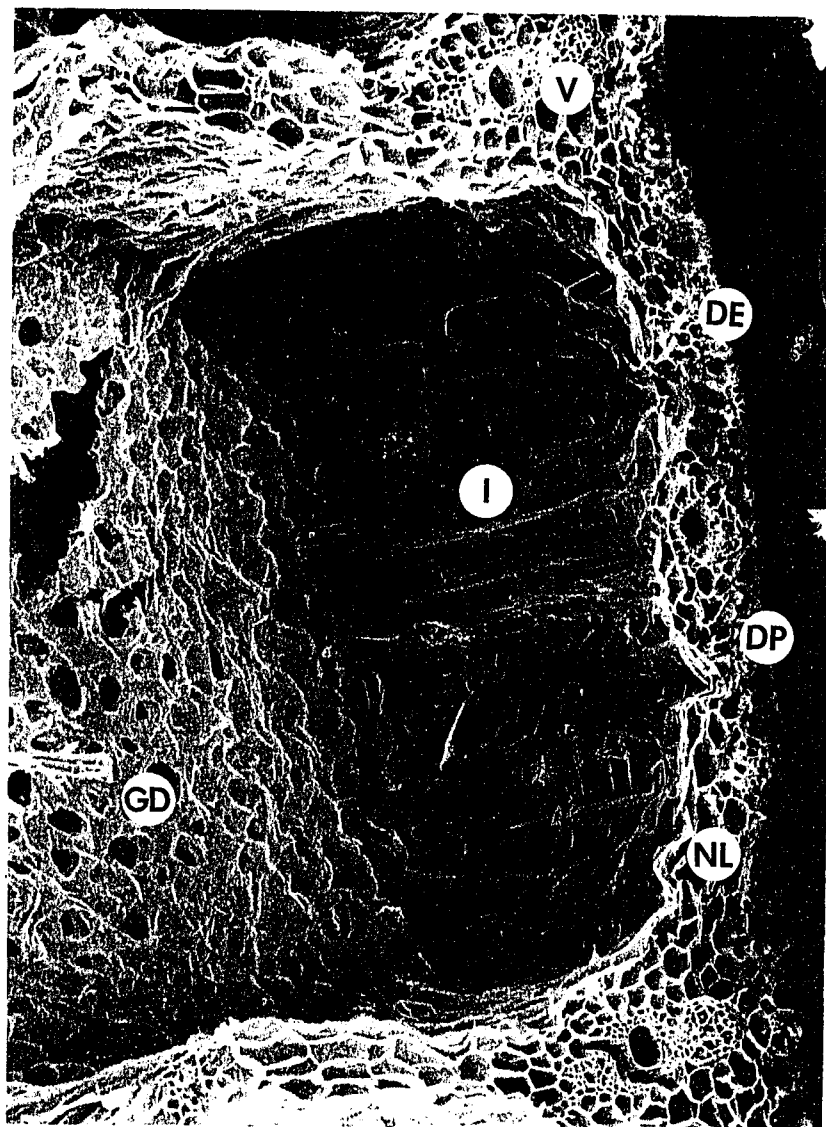


Figure 3.7. Scanning electron micrograph of *Typha latifolia* L. leaf cross-section showing AD internal surface layer (X92.5). Legend: DE, adaxial epidermis; DP, adaxial palisade; V, vascular bundle; NL, non-chlorophyllous cell layer; I, internal cell layer; GD, aerenchyma gas space diaphragm.

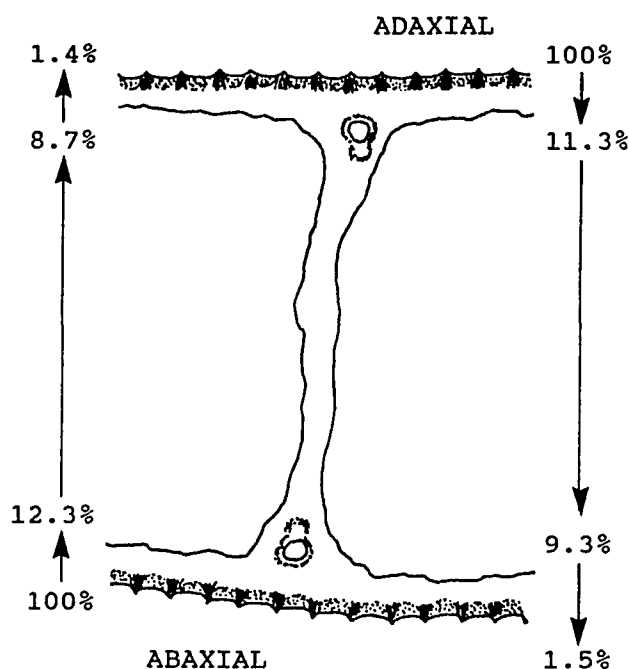


Figure 3.8. PPFD transmittance through the adaxial palisade (open bars) and abaxial palisade (closed bars) of *Typha latifolia* L. leaves. PPFD incident on the palisade was $1428 \pm 1 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$. Values are means \pm SE ($n = 8$).

entire leaf. Similarly, $176 \pm 5 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of the PPFD incident on the AB epidermis passed through the AB palisade, $124 \pm 8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ reached the internal surface of the AD palisade, and $21 \pm 1.1 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ was transmitted entirely through the leaf.

DISCUSSION

Many C_3 crop species occur in high PPFD environments and have amphistomatous leaves with a single mesophyll composed of well developed palisade and spongy parenchyma (Mott and O'Leary 1984). The cattail leaf, however, has two independent palisade layers, separated by a broad aerenchyma gas space (Fig. 3.5A). Therefore, the leaf is superficially amphistomatous, but its functional structure is better described as a pair of hypostomatous leaves joined at the edges. The relative and absolute gas exchange responses to PPFD of the AD and AB palisades were determined to understand the contribution of each palisade to total leaf gas exchange.

Intact leaves had a higher PPFD saturation point and greater maximum P_n when illuminated on the AD palisade than when illuminated on the AB palisade. The gas streams circulating over the AD and AB palisades were mixed in these measurements and, therefore, P_n reflected total P_n of both palisades. In intact leaves P_n of the AB palisade saturated at a lower PPFD than the AD palisade. This is

possibly due to lower conductance of the AB palisade (Fig. 3.2B), as the chlorophyll content of the two palisades were similar. The different gas exchange response to PPFD between the two surfaces could be influenced by differences in (1) final stomatal aperture under identical environmental conditions (Raschke et al. 1978; Pemadasa 1979), (2) photosynthetic capacity, or (3) the effect of the aerenchyma gas space $[CO_2]$ on the palisade intercellular $[CO_2]$ (C_i). At higher PPFDs, leaf conductance increased without a significant increase in P_N . This response may be caused by a continued stomatal opening in response to higher PPFDs although P_N is PPFD saturated. This response is different from the close association between conductance and P_N found in other studies (Björkman 1981). Jarvis and Mansfield (1980) found the light saturation point of conductance and P_N could differ with conductance saturating at a lower PPFD than P_N . The high variability in cattail conductance at higher PPFDs could be caused by variation in PPFD intercepted during leaf development for the different leaves. In amphistomatous leaves of crop species with a single palisade, lower P_N and conductance of the AB surface relative to the AD surface is well established (Aston 1978; Raschke et al. 1978) and reflects differences in both stomatal density and biochemical characteristics of the palisade and spongy parenchyma (Terashima and Inoue 1984, 1985).

The contributions of illuminated and shaded palisades to P_N of the total leaf (Fig. 3.2) can be evaluated from the individual responses of these palisades (Fig. 3.3). P_N by the illuminated palisade was more than 90% of total leaf P_N at PPFDs below $930 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (Figs. 3.3A and C). At PPFDs greater than $930 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, P_N of the illuminated palisade was PPFD saturated, while P_N of the shaded palisade continued to increase (Figs. 3.3A and C). The increase in P_N of the shaded palisade is due to PPFD transmitted through the illuminated palisade. The shaded palisade P_N is greater than 15% of total leaf P_N at the highest PPFDs.

Vogelmann et al. (1989) found that 10% of 450 nm or 660 nm light was transmitted entirely through Medicago leaves, about the same as the 12% PPFD transmitted through a cattail palisade (Fig. 3.8). The structure of the cattail leaf appears optimal for PPFD transmission through the palisade (Fig. 3.5). The combination of optically clear vascular bundles and the lack of intercellular gas spaces between the cells of the internal surface are factors that favor light scattering towards the leaf interior (Knapp et al. 1988; McClendon 1987; Vogelmann 1989). These features could increase PPFD transmission through the illuminated palisade of a cattail leaf relative to a "typical" C_3 leaf.

In these laboratory measurements, PPFD reaching the shaded palisade was primarily PPFD transmitted through the illuminated palisade as PPFD incident on the epidermis of the shaded palisade was less than $3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Direct PPFD normal to the soil surface in natural stands of cattail frequently exceeds $1500 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for several hours per day (Chapter 2). Although the leaves are vertically oriented, PPFD normal to the leaf surface can exceed $930 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at midday (Appendix B), a value greater than the PPFD necessary to saturate P_n found here (Figs. 3.2 and 3.3). As PPFD incident on the leaf surface can increase at lower solar angles, at least one palisade of the cattail leaf could intercept direct PPFD and remain PPFD saturated for much of the day. In the field at 50 cm below the top of the canopy, indirect PPFD measured on the shaded palisade can be $200 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or $11.6 \pm 0.7\%$ (mean \pm SE, $n = 10$) of direct PPFD measured on the illuminated palisade, a value well below PPFD saturation (J. Constable, unpublished data). If 9% of PPFD incident on the illuminated palisade is transmitted to the shaded palisade (Fig. 3.8), then at the highest PPFDs, transmitted PPFD could increase total PPFD intercepted by the shaded palisade by as much as $160 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and increase shaded palisade P_n (Figs. 3.3A and C). In these conditions shaded palisade PPFD could approach 20% of that incident on the illuminated palisade.

The high $[\text{CO}_2]$ in the aerenchyma gas space could serve as a CO_2 source for photosynthesis (Chapter 2). The AB palisade was dissected away from part of the leaf and gas exchange across the epidermis of the AD palisade was measured with a $[\text{CO}_2]$ of $350 \mu\text{L}\cdot\text{L}^{-1}$ circulating over both the epidermal and internal surfaces. This approach was used to eliminate the potential confounding effect of aerenchyma gas space CO_2 on P_{N} . The dissected AD palisade had marginally lower absolute rates of P_{N} and conductance than the intact AD palisade, but the response to PPFD was qualitatively similar (Figs. 3.2 and 3.4). The $[\text{CO}_2]$ in the aerenchyma gas spaces of the intact leaves should have been much higher than the $350 \mu\text{L}\cdot\text{L}^{-1}$ used with dissected leaves in this experiment. It appeared that the lower aerenchyma gas space $[\text{CO}_2]$ did not alter P_{N} across the AD epidermis to any great degree. The structural similarity of the AD and AB palisades indicates that P_{N} for the AB palisade would respond similarly.

The structure of the internal cells suggests that there is not a gaseous pathway between the palisade and the aerenchyma gas space (Figs. 3.5 and 3.7). If this interpretation is correct then the conductance to CO_2 (or any gas) through the internal surface should be relatively lower than through the epidermal surface (Nobel 1991). Therefore, it appears that CO_2 in the aerenchyma gas space may make a limited contribution to total leaf carbon

fixation. The P_n response of the AD and AB palisades to aerenchyma gas space $[CO_2]$ requires a more thorough evaluation, however, before definitive conclusions can be reached.

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CHAPTER 4

Gas Exchange of Typha latifolia L. Leaves at Different CO₂ Concentrations

INTRODUCTION

Cattail (Typha latifolia L.), like many emergent wetland plant species, possesses aerenchyma tissue which forms a pathway for gas transport between the submerged rhizome and the emergent leaves (Chapter 2). At dawn the CO₂ concentration ([CO₂]) in the leaf aerenchyma gas space can be as high as 6,000 $\mu\text{L}\cdot\text{L}^{-1}$ and although the [CO₂] declines during the day it is still higher than is normally found in the intercellular spaces of leaves from plants that lack an aerenchyma system (Chapter 2; Yoshie 1986). The presence of methane in the aerenchyma gas space (Appendix A) indicates that gases in the aerenchyma gas space originate from both plant and sediment processes. The elevated aerenchyma gas space [CO₂] raises the CO₂/O₂ ratio as high as 14 times that found in the atmosphere. This could increase the net photosynthetic CO₂ uptake rate (P_N) by reducing photorespiration in cattail (Ogren 1984). The impact of the aerenchyma gas space [CO₂] on P_N should vary during the day since the aerenchyma gas space [CO₂] has a distinct diurnal pattern (Chapter 2).

In leaves of plants without aerenchyma, CO_2 for photosynthesis is supplied by diffusion from the atmosphere to the photosynthetic palisade. P_N from the atmosphere is largely under stomatal control as the atmospheric $[\text{CO}_2]$ is relatively constant (Nobel 1991). In cattail, a second CO_2 source could also exist as CO_2 may diffuse from the aerenchyma gas space into the surrounding adaxial and abaxial palisades. The lack of openings comparable to stomata in the internal palisade surface facing the aerenchyma gas space (Chapter 3) argues conductance along this pathway should be constant and P_N from the aerenchyma gas space should be controlled by aerenchyma gas space $[\text{CO}_2]$. Uptake of CO_2 originating from the internal gas space of stems could be an important CO_2 source for photosynthesis prior to leaf development (Billings and Godfrey 1967) and may also increase plant water use efficiency (Osmond et al. 1987). However, the possible role of internal gas spaces in leaves has not been investigated.

The general goal of this study was to determine the effect of atmospheric $[\text{CO}_2]$ on P_N of intact cattail leaves and the effect of aerenchyma gas space $[\text{CO}_2]$ on P_N of dissected cattail palisades. P_N of intact leaves was first measured at different atmospheric $[\text{CO}_2]$ s. P_N across the epidermal surface was also measured on dissected leaves where the aerenchyma gas space $[\text{CO}_2]$ was held constant and

the epidermal $[CO_2]$ varied. Finally, P_n across the internal surface of dissected palisades was measured with the epidermal $[CO_2]$ held constant and the aerenchyma gas space $[CO_2]$ varied. The possible benefits of an elevated aerenchyma gas space $[CO_2]$ for cattail growth and reproduction were evaluated based on the results of these measurements.

MATERIALS AND METHODS

Mature plants of T. latifolia L. (cattail) used in this study were collected and cultured as previously described (Chapter 3). Gas exchange rates were measured at different $[CO_2]$ s for intact leaves and isolated palisades. Measurements of P_n for intact leaves were made as previously described (Chapter 3), except that PPFD was held constant at $1150 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and $[CO_2]$ was varied between approximately $100 \mu\text{L}\cdot\text{L}^{-1}$ and $900 \mu\text{L}\cdot\text{L}^{-1}$. Gas exchange rates across the epidermal and internal surfaces of the isolated adaxial (AD) palisade were measured in a split-chamber configuration of the clamp-on cuvette (Chapter 3) on leaves with a portion of the abaxial (AB) palisade removed by dissection. The dissection permitted control of the $[CO_2]$ in the exposed aerenchyma gas space and measurement of P_n across the internal surface. The dissection had little effect on leaf water balance, and epidermal P_n at different

PPFDs is similar for AD palisades of intact and dissected leaves (Chapter 3).

The $[CO_2]$ over the epidermal and internal surfaces of dissected leaves was independently controlled with two gas mixing systems. CO_2 -free air and 1% CO_2 were mixed for the epidermal surface using proportional gas controllers (Chapter 3) and for the internal surface with the second proportional gas controller system (model B1-2-DP, Bingham Interspace Company, Hyde Park, UT). Water vapor concentration in the gas stream for each surface was controlled as described in Chapter 3. Separate mass flow meters and water manometers were placed before the cuvette in each gas line to ensure similar flow rates and pressures over the two leaf surfaces (Fig. 4.1). Exchange rates of CO_2 and water vapor were calculated after von Caemmerer and Farquhar (1981).

The split-chamber cuvette configuration was checked for leakage between chambers covering the epidermal and internal surfaces. CO_2 -free air was passed over the epidermal surface while a $900 \mu L \cdot L^{-1}$ CO_2 gas stream was passed over the internal surface. At saturating PPFD, there was no detectable increase in the $[CO_2]$ of the gas stream leaving the epidermal cuvette. The same results were obtained when the internal cuvette was slightly pressurized.

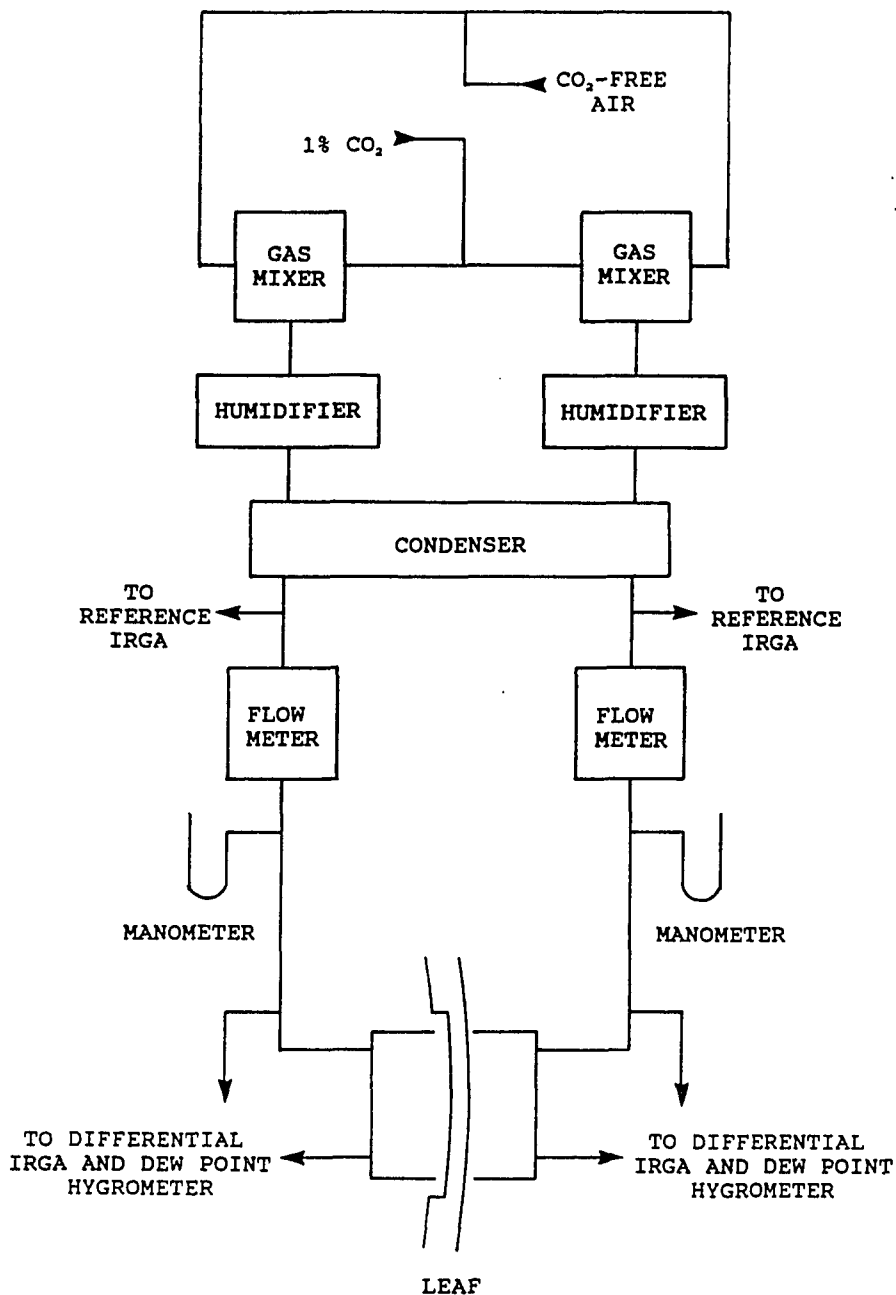


Figure 4.1. General schematic of the gas exchange system showing twin gas mixing systems and flow arrangement for the split-chamber cuvette.

Preliminary experiments determined that P_N of the AD palisade was greater than the AB palisade (Chapter 3). Therefore, measurements of P_N across the epidermal and internal surfaces focused on the AD palisade. The structure of the internal surface following dissection prevented accurate calculation of the intercellular $[CO_2]$ (C_i) and conductance to water vapor or CO_2 . The standard calculation of C_i cannot be accurately applied because (1) the internal surface area produced by dissection is uneven and is approximately 4 - 5 times greater than the smooth epidermal surface; and (2) the calculation is designed for gaseous diffusion through stomata, not the aqueous pathway that appears to occur across the internal surface (Chapter 3).

The effect of O_2 concentration on epidermal P_N was measured to estimate the relative conductance of the internal surface relative to the epidermal surface. Epidermal P_N was measured in air (21% O_2) and N_2 (1% - 1.5% O_2) using the split-chamber cuvette to estimate the relative barrier to gas flux of the internal surface. The $[CO_2]$ s on the epidermal and internal surfaces of dissected leaves were held at approximately $350 \mu L \cdot L^{-1}$ and $900 \mu L \cdot L^{-1}$, respectively in these experiments. P_N of the epidermal surface was measured with the CO_2 adjacent to the epidermal and/or internal surfaces mixed in either air (21% O_2) or N_2 (1% - 1.5% O_2).

RESULTS

In intact leaves, P_N increased 18-fold as atmospheric $[CO_2]$ was increased from about $100 \mu L \cdot L^{-1}$ to about $900 \mu L \cdot L^{-1}$ (Fig. 4.2A). Results are presented as a function of atmospheric $[CO_2]$ because the assumptions for calculation of C_i (von Caemmerer and Farquhar 1981; Nobel 1991) are not valid due to the presence of CO_2 in the aerenchyma gas space. Conductance declined 44% over the same range of atmospheric $[CO_2]$ s (Fig. 4.2B). P_N was not clearly saturated at approximately $900 \mu L \cdot L^{-1}$, the maximum $[CO_2]$ obtainable in this gas exchange system.

The response of P_N to atmospheric $[CO_2]$ across the epidermal surface of the dissected AD palisade was similar to the response of the intact leaf (Fig. 4.3A). For these measurements, the atmospheric $[CO_2]$ was varied and the $[CO_2]$ adjacent to the internal surface was held constant at either approximately $355 \mu L \cdot L^{-1}$ or $930 \mu L \cdot L^{-1}$. While the CO_2 compensation point of dissected leaves cannot be accurately determined from these data, it appears considerably greater than in intact leaves (Figs. 4.2A and 4.3A). Increasing the aerenchyma gas space $[CO_2]$ from about $355 \mu L \cdot L^{-1}$ to about $930 \mu L \cdot L^{-1}$ appeared to slightly reduce both epidermal P_N and conductance (Figs. 4.3).

P_N across the internal surface was measured at an epidermal $[CO_2]$ of approximately $350 \mu L \cdot L^{-1}$, and variable $[CO_2]$ adjacent to the internal surface (Fig. 4.4). The

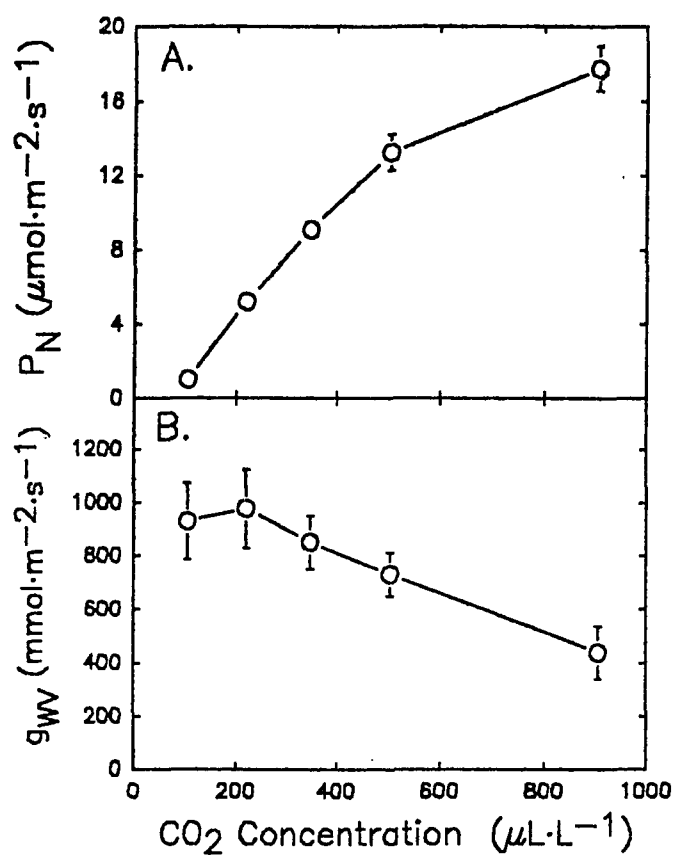


Figure 4.2. P_N (A.) and conductance (B.) at different atmospheric $[\text{CO}_2]$ s for intact *Typha latifolia* L. leaves. Values are means \pm SE ($n = 4 - 8$).

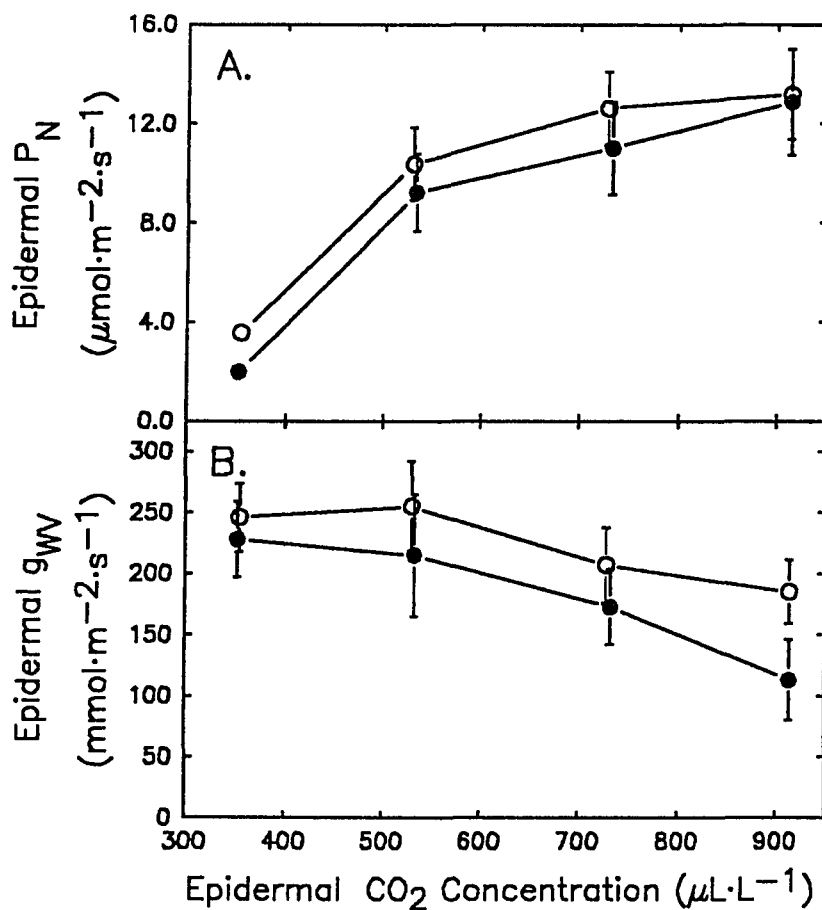


Figure 4.3. Epidermal P_N (A.) and conductance (B.) at different epidermal $[\text{CO}_2]$ s for dissected *Typha latifolia* L. leaves in the split-chamber cuvette. Aerenchyma gas space $[\text{CO}_2]$ was 355 $\mu\text{L}\cdot\text{L}^{-1}$ (open symbols) or 930 $\mu\text{L}\cdot\text{L}^{-1}$ (closed symbols). Values are means \pm SE ($n = 4-10$).

internal P_N was negligible ($0.05 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) at an aerenchyma gas space $[\text{CO}_2]$ of about $320 \mu\text{L}\cdot\text{L}^{-1}$. However, as aerenchyma gas space $[\text{CO}_2]$ increased, internal P_N increased in a linear manner reaching a maximum value of $1.92 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the highest $[\text{CO}_2]$. The CO_2 compensation point for internal P_N appeared to be over $300 \mu\text{L}\cdot\text{L}^{-1}$.

To estimate the relative conductance to gas transfer of the internal surface relative to the epidermal surface epidermal P_N was measured at a $[\text{CO}_2]$ of about $350 \mu\text{L}\cdot\text{L}^{-1}$ on the epidermis and about $900 \mu\text{L}\cdot\text{L}^{-1}$ on the internal surface in either air (21% O_2) or N_2 (1.0 - 1.5% O_2) (Table 4.1). When CO_2 adjacent to both surfaces was mixed in N_2 the maximal measured epidermal P_N was $3.03 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This maximum epidermal P_N was reduced 36% when the epidermal CO_2 was mixed in air, while the internal CO_2 remained mixed in N_2 . However, the maximum epidermal P_N was reduced only 11% when the internal CO_2 was mixed in air, while the epidermal CO_2 remained mixed in N_2 . When CO_2 was mixed in air on both the epidermal and internal surfaces epidermal P_N declined 48% to $1.6 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

The effect of varying the aerenchyma gas space $[\text{CO}_2]$ on epidermal P_N and conductance was also measured with the epidermal $[\text{CO}_2]$ held constant at $350 \mu\text{L}\cdot\text{L}^{-1}$ (Fig. 4.5). As the aerenchyma gas space $[\text{CO}_2]$ increased from about $320 \mu\text{L}\cdot\text{L}^{-1}$ to about $900 \mu\text{L}\cdot\text{L}^{-1}$, epidermal P_N decreased 69%, and

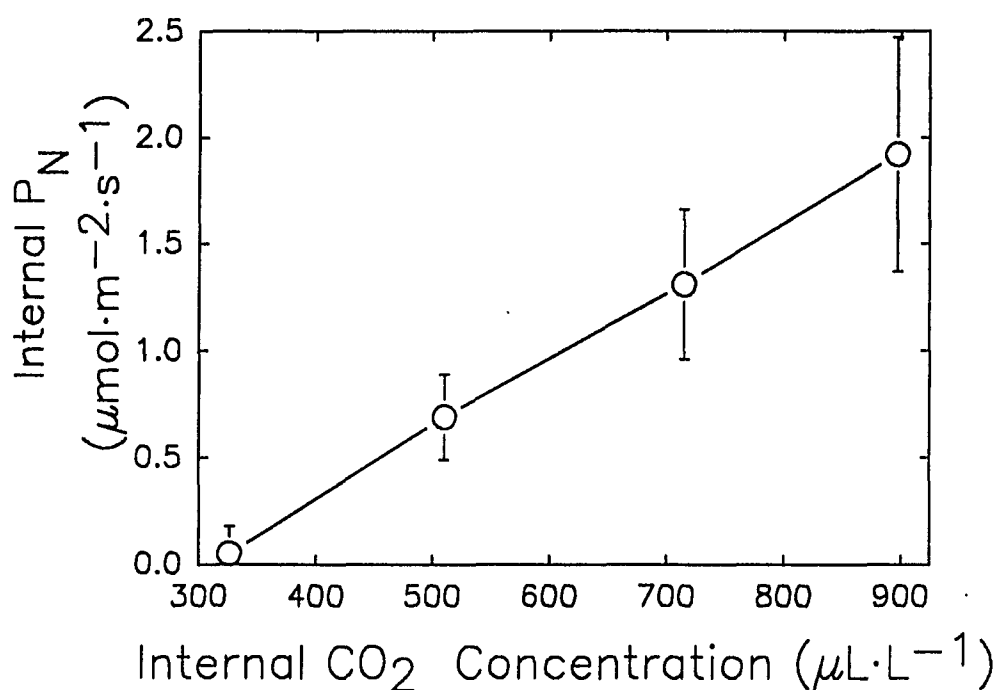


Figure 4.4. Internal P_N at different aerenchyma gas space $[CO_2]$ s for dissected *Typha latifolia* L. leaves in the split-chamber cuvette. The epidermal $[CO_2]$ was approximately $350 \mu L \cdot L^{-1}$. Values are means \pm SE ($n = 6$).

Table 4.1. Effect of air (21% O₂) and N₂ (1 - 1.5% O₂) on epidermal P_N for dissected *Typha latifolia* L. leaves in the split-chamber cuvette. Epidermal and aerenchyma gas space [CO₂] were held at 350 $\mu\text{L}\cdot\text{L}^{-1}$ and 900 $\mu\text{L}\cdot\text{L}^{-1}$ respectively and mixed in air or N₂. In each row, means with different letters indicate significant differences with an LSD test ($p \leq 0.05$). Values are means \pm SE ($n = 6$).

Treatment		Epidermal P _N $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$
<u>Epidermis</u>	<u>Aerenchyma</u>	
350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂ in N ₂	900 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂ in N ₂	3.0 \pm 0.3 a
350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂ in N ₂	900 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂ in air	2.7 \pm 0.3 ab
350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂ in air	900 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂ in N ₂	1.9 \pm 0.2 bc
350 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂ in air	900 $\mu\text{L}\cdot\text{L}^{-1}$ CO ₂ in air	1.6 \pm 0.3 c

epidermal conductance declined 31% (Figs. 4.5A and 4.5B). The maximum epidermal P_N was $4.3 \pm 0.9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, when the aerenchyma gas space $[\text{CO}_2]$ was approximately zero $\mu\text{L}\cdot\text{L}^{-1}$.

DISCUSSION

The response of the intact leaf to atmospheric $[\text{CO}_2]$ demonstrates a significant departure from that expected for a C_3 species (Fig. 4.2A). Initially the P_N response to atmospheric $[\text{CO}_2]$ is similar to other C_3 species indicating a CO_2 limitation. However, P_N continues to rise as the atmospheric $[\text{CO}_2]$ rises above the CO_2 saturation point found in other C_3 species. Sage et al. (1989) measured the response of P_N to $[\text{CO}_2]$ in several C_3 species grown at 300 $\mu\text{L}\cdot\text{L}^{-1}$ CO_2 and demonstrated that photosynthesis saturated at a C_i between 450 - 600 $\mu\text{L}\cdot\text{L}^{-1}$, a phenomenon clearly not seen here. The absence of CO_2 saturation within the range of $[\text{CO}_2]$ s used suggests cattail could have a high P_N capacity and use the very high $[\text{CO}_2]$ found in the aerenchyma gas space (Chapter 2). However, P_N of intact leaves was not excessive, even at elevated atmospheric $[\text{CO}_2]$ s (Fig. 4.2A), perhaps influenced by CO_2 in the aerenchyma gas space. The rate at which atmospheric CO_2 is taken up by intact leaves represents one of two possible CO_2 sources for cattail. The use of dissected cattail leaves allowed measurement of P_N from the aerenchyma gas space and its effect on CO_2 uptake from the atmosphere.

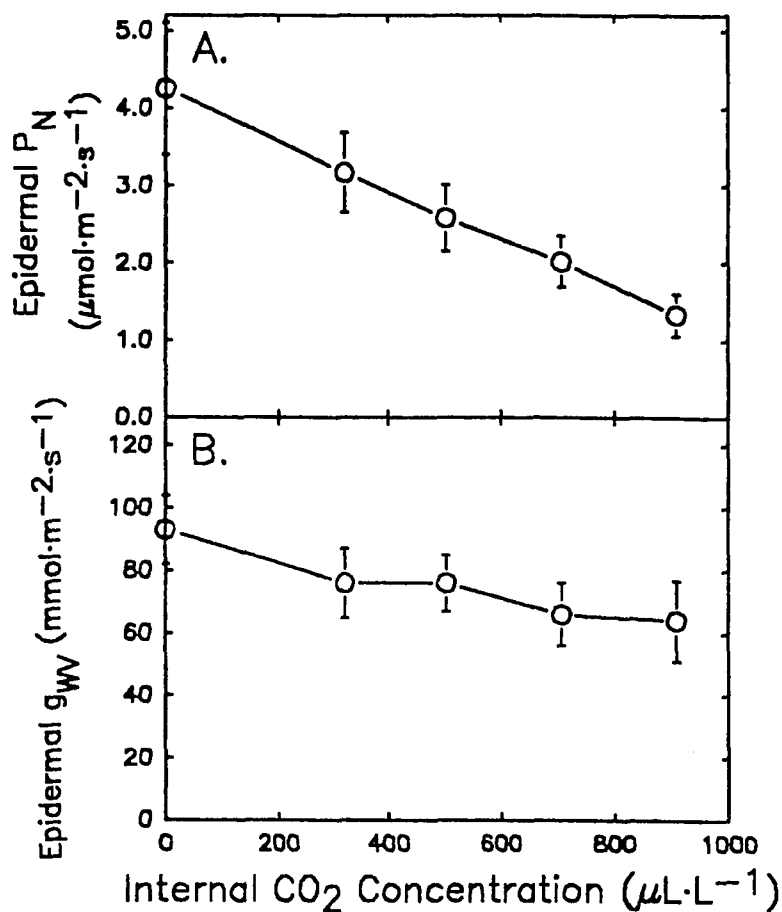


Figure 4.5. Epidermal P_N (A.) and conductance (B.) at different aerenchyma gas space [CO₂]s for dissected Typha latifolia L. leaves in the split-chamber cuvette.

Epidermal P_N of dissected leaves at different atmospheric $[CO_2]$ s was qualitatively very similar to that of intact leaves (Figs. 4.2 and 4.3). Epidermal P_N was lower at the higher aerenchyma gas space $[CO_2]$ (Fig. 4.3A). The differences were not large, but consistent, suggesting that $[CO_2]$ present in the aerenchyma gas space influenced epidermal P_N . More conclusive were direct measurements of P_N across the internal surface from the aerenchyma gas space (Fig. 4.4). Diffusion of CO_2 through the internal surface does occur and at higher aerenchyma gas space $[CO_2]$ s, internal P_N can approach $2 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Assuming a linear increase with increasing aerenchyma gas space $[CO_2]$, P_N could be as significant as epidermal P_N at the highest aerenchyma gas space $[CO_2]$ s found in the field (Chapter 2).

The ease by which oxygen diffuses to the photosynthetic cells across the internal surface was evaluated relative to the epidermal surface by measuring epidermal P_N when delivering CO_2 to each surface with either air (21% O_2) or N_2 (1 - 1.5% O_2). Based on the effect of O_2 on epidermal P_N , the conductance to O_2 flux across the internal surface appears to be approximately one third that through the epidermal surface (Table 4.1). Internal surface conductance was also estimated from gas exchange measurements of water vapor loss taking into account the greater surface area of the internal surface relative to the epidermal surface. Internal surface

conductance was $80 - 110 \text{ mmol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$, again about one third those measured for the epidermal surface (Fig. 4.3B). The measured conductance of the internal and epidermal surfaces correspond with the differing anatomical structure of the two surfaces (Chapter 3) and could explain the differences in P_N response to $[\text{CO}_2]$ of the two surfaces. Therefore, it appears a gaseous conductance pathway occurs across the epidermal surface, while an aqueous pathway occurs across the internal surface.

Epidermal P_N and conductance were inversely correlated with the $[\text{CO}_2]$ in the aerenchyma gas space (Fig 4.5). The increase in internal P_N and the decrease in epidermal P_N with increasing aerenchyma gas space $[\text{CO}_2]$ shows that the palisade can fix CO_2 from both the atmosphere and the aerenchyma gas space and that the two sources may be "competitive."

The confounding effect of CO_2 in the aerenchyma gas space was eliminated when the aerenchyma gas space $[\text{CO}_2]$ was held at about zero $\mu\text{L} \cdot \text{L}^{-1}$. At an epidermal $[\text{CO}_2]$ of about $350 \mu\text{L} \cdot \text{L}^{-1}$, the epidermal P_N was $4.3 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ and the C_i was calculated to be $287 \pm 5 \mu\text{L} \cdot \text{L}^{-1}$ (Fig. 4.5). This should be an accurate measure of the maximum palisade P_N (at an epidermal $[\text{CO}_2]$ of approximately $350 \mu\text{L} \cdot \text{L}^{-1}$) and an accurate calculation of C_i because all CO_2 originates from the epidermal gas stream. Increasing the aerenchyma gas space $[\text{CO}_2]$ clearly causes epidermal P_N to decline,

consistent with the idea that CO_2 diffusion across the internal surface is supplementing CO_2 diffusion across the epidermis. Therefore, the sum of epidermal P_N and internal P_N (total palisade P_N) should at least approach this maximum palisade P_N of $4.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at all internal $[\text{CO}_2]\text{s}$. When the measurements are summed, however (Table 4.2), total palisade P_N is approximately 30% below the expected $4.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at aerenchyma gas space $[\text{CO}_2]\text{s}$ above zero $\mu\text{L}\cdot\text{L}^{-1}$. We attribute this difference to leakage of CO_2 from the more concentrated gas stream in the aerenchyma gas space into the less concentrated epidermal gas stream, thus reducing the CO_2 differential between ingoing and outgoing epidermal gas streams and lowering epidermal P_N . Assuming a CO_2 differential for the epidermal surface of $1.0 \mu\text{L}\cdot\text{L}^{-1}$ greater than measured (within the error for the IRGA in preliminary tests), the discrepancy between the total palisade P_N and the expected maximum value of $4.3 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ would be explained. Note that extrapolation back to an aerenchyma gas space $[\text{CO}_2]$ of zero $\mu\text{L}\cdot\text{L}^{-1}$ on Fig. 4.4 produces a value of $-0.9 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ or a CO_2 efflux into the aerenchyma gas space. This is consistent with there being a small leak of CO_2 between the epidermal and aerenchyma gas space chambers of the split-chamber cuvette.

P_N across the internal surface (aerenchyma gas space CO_2) is approximately equal to P_N across the epidermis

Table 4.2. Internal P_N (from Fig. 4.4) and epidermal P_N (from Fig. 4.5A) and their sum (total P_N) for dissected Typha latifolia L. leaves at different aerenchyma gas space $[CO_2]_s$. * Extrapolated value.

P_N ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	Aerenchyma Gas Space $[CO_2]$ ($\mu\text{L}\cdot\text{L}^{-1}$)				
	0	321	503	708	908
Internal P_N	-0.9*	0.1	0.7	1.3	1.9
Epidermal P_N	4.3	3.1	2.6	2.0	1.4
Total P_N	3.4	3.2	3.3	3.3	3.3

(atmospheric CO_2) at an aerenchyma gas space $[\text{CO}_2]$ of about $825 \mu\text{L}\cdot\text{L}^{-1}$ (Fig. 4.4) and an atmospheric $[\text{CO}_2]$ of $350 \mu\text{L}\cdot\text{L}^{-1}$ (Fig. 4.5). P_N across the internal surface would therefore be higher than P_N from across the epidermal surface at aerenchyma gas space $[\text{CO}_2]$ s greater than $825 \mu\text{L}\cdot\text{L}^{-1}$.

Cattail internal P_N is slightly lower than the highly variable P_N from the internal gas space in the stem of Eriogonum inflatum, although the internal $[\text{CO}_2]$ s reach similar levels (Osmond et al. 1987). Internal P_N in cattail is also slightly below that occurring in the internal gas space of the young pods of Isomeris arborea, a desert shrub that has a gas space $[\text{CO}_2]$ between $500 \mu\text{L}\cdot\text{L}^{-1}$ and $3,000 \mu\text{L}\cdot\text{L}^{-1}$ (Goldstein et al. 1991).

Elevated atmospheric $[\text{CO}_2]$ affects processes other than P_N including dark respiration (Amthor et al. 1992) and both uptake and reduction of NO_3^- (Pace et al. 1990). Increases in dry weight (Larigauderie et al. 1988) and total seed biomass (Zangerl and Bazzaz 1984) are among the many growth responses to elevated $[\text{CO}_2]$. If a similar response occurs when CO_2 is present in the aerenchyma gas space, cattail metabolism could be altered. Appreciable carbon gain by the AD and AB palisades may only occur at aerenchyma gas space $[\text{CO}_2]$ s greater than $900 \mu\text{L}\cdot\text{L}^{-1}$ and therefore limit carbon gain to 2 - 3 hours per day. However, even when the aerenchyma gas space $[\text{CO}_2]$ is too

low to permit carbon gain there could still be an effect on cattail. Elevated atmospheric $[CO_2]$ lowers the rate of dark respiration, (Gifford et al. 1985; Amthor et al. 1992), but is not associated with a reduction in relative growth rate (Bunce and Caulfield 1991). In cattail, a similar phenomenon could occur for cells of the internal surface and conserve a small amount of carbon that could be re-allocated to other metabolic processes.

The CO_2 found in the aerenchyma gas space can be assimilated photosynthetically and increase P_n of the palisade. Carbon gained through enhanced photosynthesis and/or reduced respiration could be allocated to growth and positively influence the competitive ability (Grace 1985) and rate of vegetative reproduction (Grace and Wetzel 1981) of cattail plants increasing the chances for ecological success.

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CHAPTER 5

Summary and Conclusions

Cattail (Typha latifolia L.) is a widely distributed emergent wetland plant species. This large plant has long almost vertically oriented leaves. There is a well developed aerenchyma gas space system that connects the gas spaces of the leaves and the submerged rhizomes. The first goal of this study was to examine the structure of the aerenchyma gas space system and the extent to which CO₂ accumulated in the aerenchyma gas space. The second goal was to examine the adaxial and abaxial leaf surfaces for differences in gas exchange response to PPFD. And the third goal was to evaluate the effect of aerenchyma gas space [CO₂] on CO₂ uptake from the atmosphere and on CO₂ uptake directly from the aerenchyma gas space.

The consistent diurnal fluctuation in aerenchyma gas space [CO₂] in the field suggests that gases rich in CO₂ produced by microbial and plant respiration diffuse to the leaf aerenchyma gas space from the rhizome and accumulate at night. In the leaf aerenchyma gas space these gases accumulate due to closed stomata and limited photosynthetic activity. After sunrise, photosynthetic uptake of aerenchyma gas space CO₂ could produce the characteristic morning decline in aerenchyma gas space [CO₂] (Chapter 2).

In C_3 species, like cattail, the photosynthetic pathway is CO_2 -limited, therefore, CO_2 present in the aerenchyma gas space has the potential to positively influence photosynthesis.

The aerenchyma gas space separates the adaxial (AD) and abaxial (AB) photosynthetic palisades, producing a leaf that is structurally similar to two hypostomatous leaves joined at the edges. As only one of the two palisades receives direct PPFD in the field at any given time, differences in their gas exchange response to PPFD could effect total leaf carbon gain. In intact leaves, between 85% and 100% of total leaf P_n occurs through the directly illuminated palisade. At the greatest PPFDs, up to 15% of total leaf P_n can be attributed to the indirectly illuminated palisade where P_n is a function of PPFD transmitted through the illuminated palisade and indirect PPFD incident on the shaded palisade epidermis. Transmitted PPFD can increase the PPFD received by the indirectly illuminated palisade by almost 50%. While the AD and AB palisades have approximately similar points of PPFD saturation, the AD palisade has 39% greater P_n and a greater conductance than the AB palisade (Chapter 3). Because of this difference the palisade receiving direct PPFD has a profound impact on total leaf P_n .

Carbon dioxide only diffuses from the atmosphere to the palisade in C_3 leaves lacking an aerenchyma gas space.

In cattail, there can be two distinct pathways for CO_2 diffusion to the palisade. The first is the gaseous pathway from the atmospheric CO_2 source through stomata, which can have a relatively high conductance. The second pathway is from the aerenchyma gas space CO_2 source through the tightly connected cells of the internal layer (Chapter 3). This pathway has a relatively lower conductance because all CO_2 must apparently diffuse through a cell wall and/or cytoplasmic solution(s) (Chapters 3 and 4). The predominant CO_2 source for photosynthesis in the early morning could be the aerenchyma gas space when the gradient in $[\text{CO}_2]$ between the mesophyll and the aerenchyma gas space is steepest. The proportion of CO_2 originating from the aerenchyma gas space would potentially decrease as the day progresses and the $[\text{CO}_2]$ gradient declines.

The cattail palisade is exposed to two CO_2 sources of differing concentration and pathway conductance, which does not occur most C_3 species. The P_n response of the intact cattail leaf to varying atmospheric $[\text{CO}_2]$ was similar to that of other C_3 species, except leaves saturated at a higher $[\text{CO}_2]$ than other C_3 species (Chapter 4). Manipulation of the $[\text{CO}_2]$ in the aerenchyma gas space using dissected leaves demonstrated that P_n from the aerenchyma gas space could occur, but at relatively low rates. As aerenchyma gas space $[\text{CO}_2]$ increased there was a linear increase in internal P_n . The highly concentrated

aerenchyma gas space CO_2 source also effected CO_2 uptake from the atmosphere and there was a notable decline in epidermal P_n as aerenchyma gas space $[\text{CO}_2]$ increased. It is probable that at the greater aerenchyma gas space $[\text{CO}_2]$ s found in the field, carbon gain from the aerenchyma gas space could be considerably greater.

During the morning when the aerenchyma gas space $[\text{CO}_2]$ is high increased CO_2 diffusion from the aerenchyma gas space could benefit cattail by reducing photorespiration and thereby increasing carbon gain. Carbon gained through increased CO_2 fixation or retained due to reduced respiratory CO_2 loss could increase cattail's competitive ability, survival and reproductive success.

APPENDIX A

Methane Concentrations in Soil and Cattail Tissues

INTRODUCTION

Methane can be used as a tracer of gas flux through aquatic plants into the atmosphere because it is produced in anaerobic sediments, but is not metabolized by plants. Methane concentrations have been measured in samples from the aerenchyma gas spaces of several wetland species including cattail (Dacey 1981; Knapp and Yavitt 1991; Sebachner et al. 1985). The concentration of methane (CH_4) in the aerenchyma of cattail was measured to determine if gases produced in the anaerobic sediments could diffuse into the aerenchyma gas space.

MATERIALS AND METHODS

Methane was collected from rhizomes and leaves in a manner identical to that used to sample $[\text{CO}_2]$ in the aerenchyma gas space of leaves (Chapter 2). Rhizome "ports" were constructed identically to leaf "ports", but with 30 cm lengths of polyethylene tubing. One week prior to sampling, plants were carefully lifted from the sediment using a spade. Underground structures were inspected for damage, washed with water, and the "port" was inserted into the rhizome aerenchyma gas space 10 cm from the rhizome-

shoot transition. The port was sealed with vacuum grease and the plant gently replaced in its original position. On 1 June 1991 samples were taken from the leaf and rhizome aerenchyma gas space as well as from the atmosphere surrounding the leaves every three hours between dawn and dusk using gas-tight syringes (Hamilton Company, Reno, NV). Syringe needle were immediately sealed by insertion into rubber stoppers and returned to the laboratory.

Methane concentration ($[\text{CH}_4]$) in each sample was determined using a gas chromatograph (model 3600, Varion Corporation, Sunnyvale, CA) equipped with a flame ionization detector (FID). Gases were separated on a Poropak-Q column (80/100 mesh, 1.8 m x 3 mm; Millipore Corporation, Woburn, MA) maintained at 80 C using Helium carrier gas ($60 \text{ ml} \cdot \text{min}^{-1}$). The FID was supplied hydrogen and air at 40 and $300 \text{ ml} \cdot \text{min}^{-1}$, respectively. The FID analog signal was digitized and calculated on an integrator (model Vista CDS 401, Varion Corporation, Sunnyvale, CA). Methane concentrations were quantified by comparing peak areas of samples and standards. Certified standards (1.0 and $100 \mu\text{L} \cdot \text{L}^{-1} \text{CH}_4$ in N_2 ; Matheson Gas Products, East Rutherford, NJ) bracketed every 15 - 20 samples.

RESULTS AND DISCUSSION

Methane is found in the aerenchyma gas space of cattail leaves and rhizomes. The diurnal $[\text{CH}_4]$ in the

aerenchyma gas space and in the atmosphere followed the same pattern as found for $[CO_2]$ where the greatest values occurred at dawn (Table A.1). The rhizome, embedded in anaerobic sediments that favor methane production, contained much greater $[CO_2]$ and $[CH_4]$ than either the atmosphere or leaf aerenchyma gas space, but had great variability (Table A.2). The concentration of methane in the rhizome increased through the day as found by Schütz et al. (1989). The presence of methane in the aerenchyma gas space supports the idea that gases produced in the anaerobic sediments can enter the aerenchyma gas space and diffuse to the cattail leaf. As methane is present in sediments at lower concentrations than CO_2 , it is probable that sediment generated CO_2 could also diffuse into the aerenchyma gas space and supplement cattail photosynthesis.

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Table A.1. Diurnal measurements of methane concentration ($[\text{CH}_4]$) in the aerenchyma gas space of Typha latifolia L. and the atmosphere outside the leaves. Values are means \pm SE (n = 5).

Time	$[\text{CH}_4]$ ($\mu\text{L} \cdot \text{L}^{-1}$)	
	Atmosphere	Leaf
6:30	4.2 \pm 0.2	7.4 \pm 1.4
9:00	3.4 \pm 0.2	4.1 \pm 0.4
12:00	3.0 \pm 0.1	3.8 \pm 0.5
15:00	2.9 \pm 0.3	3.7 \pm 0.7
17:30	2.8 \pm 0.2	3.7 \pm 0.5
19:50	3.4 \pm 0.2	6.5 \pm 1.7

Table A.2. Diurnal measurements of CO_2 concentration ($[\text{CO}_2]$) and CH_4 concentration ($[\text{CH}_4]$) in the aerenchyma gas space of Typha latifolia L. rhizomes. Values are means \pm SE (n = 4).

Time	Rhizome Gas ($\mu\text{L} \cdot \text{L}^{-1}$)	
	$[\text{CO}_2]$	$[\text{CH}_4]$
6:30	1766 \pm 667	535 \pm 456
9:00	4035 \pm 1127	1456 \pm 778
12:00	4209 \pm 1422	1657 \pm 908
15:00	4813 \pm 1838	1755 \pm 1007
17:30	4997 \pm 2562	1767 \pm 1292
19:50	7161 \pm 2543	1862 \pm 1211

APPENDIX B

Field Measurements of Leaf Pressurization

INTRODUCTION

Movement of gases within the aerenchyma gas space was once thought to occur by diffusion along partial pressure gradients (Higuchi 1982). More recent studies have identified movement by mass flow mechanisms in several species (Dacey 1981, 1987; Grosse et al. 1991). The pressure of the aerenchyma gas space of cattail was measured relative to atmospheric pressure to determine if a pressure gradient, sufficient to produce mass flow, existed between young and old leaves.

MATERIALS AND METHODS

Pressure in the aerenchyma gas space was measured in younger (leaf numbers 9 or 10) and older (leaf numbers 4 or 5) Typha latifolia L. leaves at a field site 1.3 km northeast of the Louisiana State University Ben Hur Research Farm in Baton Rouge, Louisiana (Chapter 2). Pressure was measured by inserting a 22-gauge stainless steel syringe needle attached to a 20-cm length of polyethylene tubing (inside diameter 0.86 mm) ending in a 3-way polyethylene valve into the leaf aerenchyma gas space approximately 120 cm above the ground. The needle was held

in place by paper tape and sealed at the site of insertion with vacuum grease. Pressure was measured relative to the atmosphere with an electronic pressure transducer (model PX160, Omega Engineering, Stamford, CT), calibrated against a water manometer. Concurrent with pressure measurements the following additional data were recorded: PPFD normal to the soil surface, measured with a photodiode (Hamamatsu Corporation, Bridgewater, NJ) calibrated against a quantum sensor (model LI-190SB, LiCor, Lincoln, NE); PPFD normal to the leaf surface, measured with a quantum sensor (LiCor, Lincoln, NE); and leaf aerenchyma gas space and air temperatures, measured with fine-wire, copper-constantan thermocouples. Output from all devices was collected every 10 s for 30 min on either side of solar noon using a datalogger (model CR-21, Campbell Scientific Company, Logan UT) hard-wired to a field portable microcomputer (model TRS-80, Tandy Corporation, Fort Worth TX).

RESULTS AND DISCUSSION

Internal pressurization may be caused by several processes in plants (See Dacey 1981 for a detailed explanation). In T. latifolia L. both young and mature leaves pressurized to approximately the same degree, although there was wide variability (Table B.1). The pressures measured here are similar to those found in Nuphar (Dacey 1981) and Nelumbo (Mevi-Schutz and Grosse

Table B.1. Leaf pressurization data sets indicating the range of aerenchyma gas space pressures measured on a specific date and its correlation with aerenchyma gas space temperature and PPFD normal to the leaf surface.

Date and Leaf Type (Y=Young; M=Mature)	Pressure Range	Correlations with leaf temperature and PPFD
July 16-Y	20 - 30 Pa	Pa= -0.1 + 4.12(Leaf Temp); r=.4690 Pa= 16.9 + 0.02(Leaf PPFD); r=.4883
July 12-Y	30 - 40 Pa	Pa= -4.6 + 1.40(Leaf Temp); r=.1850 Pa= 34.4 + 0.01(Leaf PPFD); r=.2840
July 12-Y	90 - 110 Pa	Pa= -0.5 + 4.36(Leaf Temp); r=.3004 Pa= 72.0 + 0.05(Leaf PPFD); r=.5362
All Dates-Y	20 - 110 Pa	Pa= -489.1 + 16.0(Leaf Temp); r=.5069 Pa= 87.9 - 0.05(Leaf PPFD); r=.3121
July 18-M	100 - 120 Pa	Pa= -143.3 + 7.68(Leaf Temp); r=.3958 Pa= 125.0 - 0.01(Leaf PPFD); r=.1354
July 15-M	70 - 80 Pa	Pa= -32.1 + 3.34(Leaf Temp); r=.3871 Pa= 66.2 + 0.02(Leaf PPFD); r=.4216
July 14-M	70 - 90 Pa	Pa= -103.9 + 5.85(Leaf Temp); r=.5940 Pa= 69.2 + 0.01(Leaf PPFD); r=.4967
July 14-M	90 - 110 Pa	Pa= -340.8 + 13.7(Leaf Temp); r=.7021 Pa= 73.0 + 0.02(Leaf PPFD); r=.7482
July 13-M	40 - 50 Pa	Pa= 23.6 + 0.80(Leaf Temp); r=.6876 Pa= 61.1 + 0.02(Leaf PPFD); r=.2513
July 10-M	80 - 90 Pa	Pa= 608.3 - 15.4(leaf Temp); r=.2396 Pa= -27.5 + 0.11(Leaf PPFD); r=.4039
July 9-M	30 - 40 Pa	Pa= 2.9 + 0.93(leaf Temp); r=.2041 Pa= 25.1 + 0.01(Leaf PPFD); r=.7167
All Dates-M	40 - 120 Pa	Pa= 61.5 + 0.04(Leaf Temp); r=.1825 Pa= 32.8 + 0.05(Leaf PPFD); r=.6232

1988), but significantly lower than in Phragmites (Armstrong and Armstrong 1991). The similar pressures of older and younger leaves makes it unlikely that a mass flow gas transport system such as found in Nuphar (Dacey 1980, 1981) and Nelumbo (Grosse et al. 1991; Mevi-Schutz and Grosse 1988) exists. When all data were combined there appeared to be no distinct relationship between leaf pressure and either leaf aerenchyma gas space temperature (Fig. B.1), or PPFD normal to the leaf surface (Fig. B.2). Clusters of points in Figures B.1 and B.2 represent individual data sets in which the relationship between leaf pressure and either leaf aerenchyma gas space temperature or PPFD normal to the leaf is somewhat clearer as estimated by least squares regressions (Table B.1). The lack of a clear relationship between leaf pressure and the variables analyzed here suggests that pressurization in cattail leaves is a complex process controlled by multiple factors.

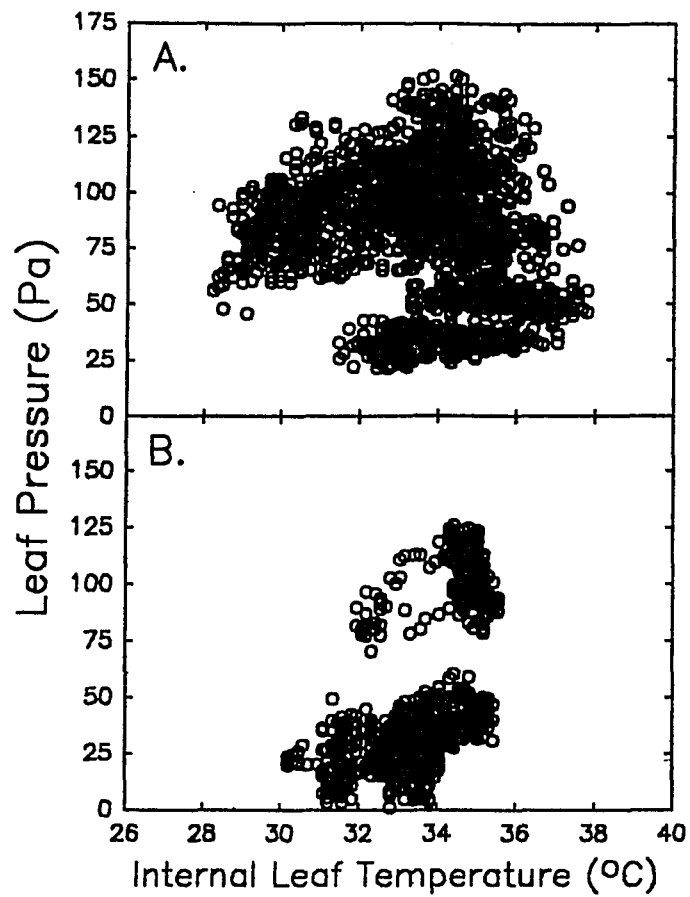


Figure B.1. Aerenchyma gas space pressures in old (A.) and young (B.) *Typha latifolia* L. leaves as a function of aerenchyma gas space temperature.

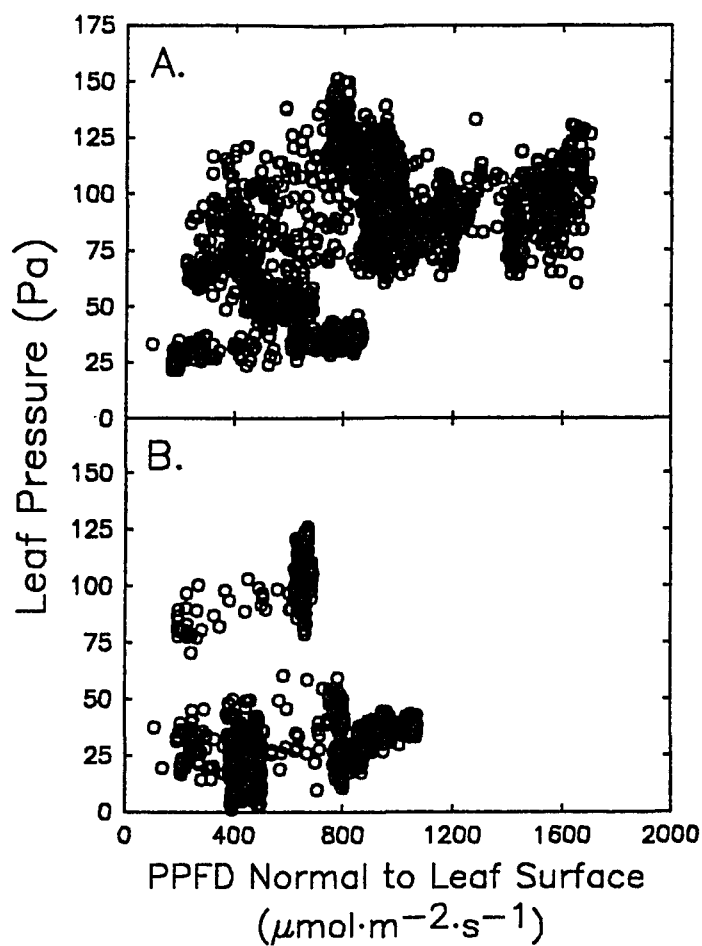


Figure B.2. Aerenchyma gas space pressures in old (A.) and young (B.) *Typha latifolia* L. leaves as a function of PPFD normal to the leaf surface.

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APPENDIX C

Tissue Volume and Conductance to Gas Flow

INTRODUCTION

The objective of these experiments was to quantify the aerenchyma volume within whole cattail plants and the size of the CO₂ reservoir. Measurements of the tissue conductance to gas flow through cattail tissues permitted a comparison of the relative conductance to gas flow within and between different portions of the plant.

MATERIAL AND METHODS

Leaf aerenchyma gas space volumes and cross-sectional areas were determined as described in Chapter 2. Tissue resistance was measured by placing 10 cm lengths of Typha latifolia L. tissue between two water manometers constructed from glass tubing. The manometers permitted a known pressure gradient to be applied through the aerenchyma gas space. Tissue resistance (R) was calculated as:

$$R \text{ (s} \cdot \text{cm}^{-2}\text{)} = \frac{(\Delta H_{mB}) - (\Delta H_{mA})}{F} \cdot \frac{1}{L} \cdot A$$

where ΔH refers to the change in water level in the ingoing manometer (mA) and outgoing manometer (mB) in cm; F is gas flow through the tissue segment calculated from the volume

change in a water-filled tube placed prior to the ingoing manometer over time in $\text{cm}\cdot\text{s}^{-1}$; L is the length of the tissue segment in cm ; and A is the cross-sectional area of the segment in cm^2 . The reciprocal value is tissue conductance to gas flow.

RESULTS AND DISCUSSION

The cross-sectional area occupied by aerenchyma gas space at the leaf base is lower in younger leaves, (higher leaf numbers), but the percentage of cross-sectional area remains relatively constant, between 50% - 60% (Fig. C1.A). This is comparable to the percentages found by Pazourek (1977). Differences in leaf base cross-sectional area of aerenchyma gas space is closely related to the total aerenchyma gas space volume of leaves in all but the oldest leaves (Fig. C.1B). The six oldest leaves contain 69% of the total aerenchyma gas space volume in cattail plants with 12 leaves. The greater volume and cross-sectional area at the leaf base of older leaves could increase CO_2 flux from the rhizome into the leaf relative to CO_2 flux into younger leaves. If the CO_2 diffusion into older leaves exceeds that into younger leaves, P_n of older mature leaves could be more significantly influenced by aerenchyma gas space $[\text{CO}_2]$ than that of younger leaves.

The tissue conductance closely corresponds to expectations based on anatomical observations (Chapter 2).

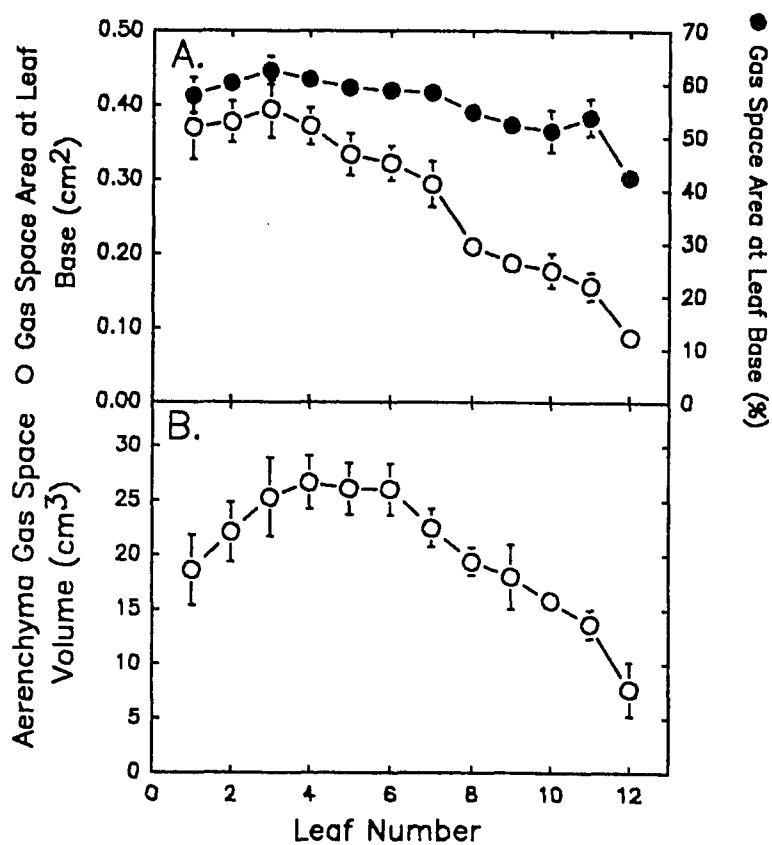


Figure C.1. A. Cross-sectional area occupied by aerenchyma gas spaces in absolute area (open symbols) and in percent (closed symbols) of total cross-sectional area at the leaf base as a function of leaf number. B. Aerenchyma gas space volume as a function of leaf number. Values are means \pm SE ($n = 3$).

While gases can move through the aerenchyma gas space in all examined segments, the lowest conductance occurs in the rhizome-shoot transition where the aerenchyma tissue is compressed by the meristem (Table C.1). In contrast, greatest conductance occurs 10 cm above the leaf base (Table C.1). Gases present in the aerenchyma gas space of the rhizome could diffuse to the leaves, with the greatest restriction to gas flux occurring in the rhizome-shoot-transition.

Table C.1. Gas flow conductance in different anatomical locations of Typha latifolia L. Values are means \pm SE.

<u>Tissue</u>	<u>Conductance (cm\cdots$^{-1}$)</u>	<u>n</u>
Rhizome	0.36 \pm 0.16	9
Rhizome-Shoot-Transition	0.05 \pm 0.01	10
Leaf (10 cm from base)	6.01 \pm 1.02	12
Leaf (45 cm from base)	1.95 \pm 0.32	12
Leaf (85 cm from base)	0.23 \pm 0.04	5

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VITA

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

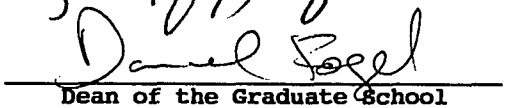
Candidate: John V.H. Constable

Major Field: Botany



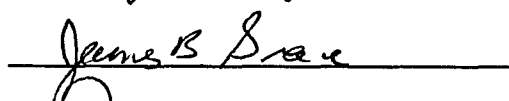
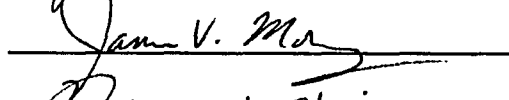
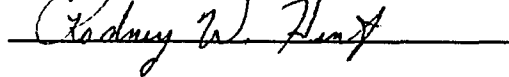
Title of Dissertation: Photosynthetic Response to Elevated CO₂ Concentrations
in the Aerenchyma of Typha latifolia L. Leaves

Approved:


Major Professor and Chairman


Dean of the Graduate School

EXAMINING COMMITTEE:

Date of Examination:

February 16, 1993